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(54) Title: COMPOSITIONS AND METHODS FOR INHIBITING EXPRESSION OF EG5 GENE

(57) Abstract: The invention relates to a double-stranded ribonucleic acid (dsRNA) for inhibiting the expression of the Eg5 gene (Eg5 gene), comprising an antisense strand having a nucleotide sequence which is less that 30 nucleotides in length, generally 19-25 nucleotides in length, and which is substantially complementary to at least a part of the Eg5 gene. The invention also relates to a pharmaceutical composition comprising the dsRNA together with a pharmaceutically acceptable carrier; methods for treating diseases caused by Eg5 expression and the expression of the Eg5 gene using the pharmaceutical composition; and methods for inhibiting the expression of the Eg5 gene in a cell.

COMPOSITIONS AND METHODS FOR INHIBITING EXPRESSION OF Eg5 GENE

Related Applications

This application claims the benefit of U.S. Provisional Application No. 60/787,762, filed March 31, 2006, and U.S. Provisional Application No. 60/870,259, filed December 15, 2006. Both prior applications are incorporated herein by reference in their entirety.

Field of the Invention

This invention relates to double-stranded ribonucleic acid (dsRNA), and its use in mediating RNA interference to inhibit the expression of the Eg5 gene and the use of the dsRNA to treat pathological processes mediated by Eg5 expression, such as cancer, alone or in combination with a dsRNA targeting vacular endothelian growth factor (VEGF).

Background of the Invention

The maintenance of cell populations within an organism is governed by the cellular processes of cell division and programmed cell death. Within normal cells, the cellular events associated with the initiation and completion of each process is highly regulated. In proliferative disease such as cancer, one or both of these processes may be perturbed. For example, a cancer cell may have lost its regulation (checkpoint control) of the cell division cycle through either the overexpression of a positive regulator or the loss of a negative regulator, perhaps by mutation.

Alternatively, a cancer cell may have lost the ability to undergo programmed cell death through the overexpression of a negative regulator. Hence, there is a need to

develop new chemotherapeutic drugs that will restore the processes of checkpoint control and programmed cell death to cancerous cells.

One approach to the treatment of human cancers is to target a protein that is essential for cell cycle progression. In order for the cell cycle to proceed from one phase to the next, certain prerequisite events must be completed. There are checkpoints within the cell cycle that enforce the proper order of events and phases. One such checkpoint is the spindle checkpoint that occurs during the metaphase stage of mitosis. Small molecules that target proteins with essential functions in mitosis may initiate the spindle checkpoint to arrest cells in mitosis. Of the small molecules that arrest cells in mitosis, those which display anti-tumor activity in the clinic also induce apoptosis, the morphological changes associated with programmed cell death. An effective chemotherapeutic for the treatment of cancer may thus be one which induces checkpoint control and programmed cell death. Unfortunately, there are few compounds available for controlling these processes within the cell. Most compounds known to cause mitotic arrest and apoptosis act as tubulin binding agents. These compounds alter the dynamic instability of microtubules and indirectly alter the function/structure of the mitotic spindle thereby causing mitotic arrest. Because most of these compounds specifically target the tubulin protein which is a component of all microtubules, they may also affect one or more of the numerous normal cellular processes in which microtubules have a role. Hence, there is also a need for small molecules that more specifically target proteins associated with proliferating cells.

Eg5 is one of several kinesin-like motor proteins that are localized to the mitotic spindle and known to be required for formation and/or function of the bipolar mitotic spindle. Recently, there was a report of a small molecule that disturbs bipolarity of the mitotic spindle (Mayer, T. U. et. al. 1999. Science 286(5441) 971-4, herein incorporated by reference). More specifically, the small molecule induced the formation of an aberrant mitotic spindle wherein a monoastral array of microtubules emanated from a central pair of centrosomes, with chromosomes attached to the distal

ends of the microtubules. The small molecule was dubbed "monastrol" after the monoastral array. This monoastral array phenotype had been previously observed in mitotic cells that were immunodepleted of the *Eg5* motor protein. This distinctive monoastral array phenotype facilitated identification of monastrol as a potential inhibitor of *Eg5*. Indeed, monastrol was further shown to inhibit the *Eg5* motor-driven motility of microtubules in an in vitro assay. The *Eg5* inhibitor monastrol had no apparent effect upon the related kinesin motor or upon the motor(s) responsible for golgi apparatus movement within the cell. Cells that display the monoastral array phenotype either through immunodepletion of *Eg5* or monastrol inhibition of *Eg5* arrest in M-phase of the cell cycle. However, the mitotic arrest induced by either immunodepletion or inhibition of *Eg5* is transient (Kapoor, T. M., 2000. J Cell Biol 150(5) 975-80). Both the monoastral array phenotype and the cell cycle arrest in mitosis induced by monastrol are reversible. Cells recover to form a normal bipolar mitotic spindle, to complete mitosis and to proceed through the cell cycle and normal cell proliferation. These data suggest that a small molecule inhibitor of *Eg5* which induced a transient mitotic arrest may not be effective for the treatment of cancer cell proliferation. Nonetheless, the discovery that monastrol causes mitotic arrest is intriguing and hence there is a need to further study and identify compounds which can be used to modulate the *Eg5* motor protein in a manner that would be effective in the treatment of human cancers. There is also a need to explore the use of these compounds in combination with other antineoplastic agents.

VEGF (also known as vascular permeability factor, VPF) is a multifunctional cytokine that stimulates angiogenesis, epithelial cell proliferation, and endothelial cell survival. VEGF can be produced by a wide variety of tissues, and its overexpression or aberrant expression can result in a variety disorders, including cancers and retinal disorders such as age-related macular degeneration and other angiogenic disorders.

Recently, double-stranded RNA molecules (dsRNA) have been shown to block gene expression in a highly conserved regulatory mechanism known as RNA

interference (RNAi). WO 99/32619 (Fire et al.) discloses the use of a dsRNA of at least 25 nucleotides in length to inhibit the expression of genes in *C. elegans*. dsRNA has also been shown to degrade target RNA in other organisms, including plants (see, e.g., WO 99/53050, Waterhouse et al.; and WO 99/61631, Heifetz et al.), *Drosophila* (see, e.g., Yang, D., et al., *Curr. Biol.* (2000) 10:1191-1200), and mammals (see WO 00/44895, Limmer; and DE 101 00 586.5, Kreutzer et al.). This natural mechanism has now become the focus for the development of a new class of pharmaceutical agents for treating disorders that are caused by the aberrant or unwanted regulation of a gene.

Despite significant advances in the field of RNAi and advances in the treatment of pathological processes mediated by Eg5 expression, there remains a need for an agent that can selectively and efficiently silence the Eg5 gene using the cell's own RNAi machinery that has both high biological activity and in vivo stability, and that can effectively inhibit expression of a target Eg5 gene for use in treating pathological processes mediated by Eg5 expression.

Summary of the Invention

The invention provides double-stranded ribonucleic acid (dsRNA), as well as compositions and methods for inhibiting the expression of the Eg5 gene in a cell or mammal using such dsRNA, alone or in combination with a dsRNA targeting VEGF. The invention also provides compositions and methods for treating pathological conditions and diseases caused by the expression of the Eg5 gene, such as in cancer. The dsRNA of the invention comprises an RNA strand (the antisense strand) having a region which is less than 30 nucleotides in length, generally 19-24 nucleotides in length, and is substantially complementary to at least part of an mRNA transcript of the Eg5 gene.

In one embodiment, the invention provides double-stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of the Eg5 gene. The dsRNA

comprises at least two sequences that are complementary to each other. The dsRNA comprises a sense strand comprising a first sequence and an antisense strand comprising a second sequence. The antisense strand comprises a nucleotide sequence which is substantially complementary to at least part of an mRNA encoding Eg5, and the region of complementarity is less than 30 nucleotides in length, generally 19-24 nucleotides in length. The dsRNA, upon contacting with a cell expressing the Eg5, inhibits the expression of the Eg5 gene by at least 40%.

For example, the dsRNA molecules of the invention can be comprised of a first sequence of the dsRNA that is selected from the group consisting of the sense sequences of Tables 1-3 and the second sequence is selected from the group consisting of the antisense sequences of Tables 1-3. The dsRNA molecules of the invention can be comprised of naturally occurring nucleotides or can be comprised of at least one modified nucleotide, such as a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, and a terminal nucleotide linked to a cholesteryl derivative. Alternatively, the modified nucleotide may be chosen from the group of: a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an abasic nucleotide, 2'-amino-modified nucleotide, 2'-alkyl-modified nucleotide, morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide. Generally, such modified sequence will be based on a first sequence of said dsRNA selected from the group consisting of the sense sequences of Tables 1-3 and a second sequence selected from the group consisting of the antisense sequences of Tables 1-3.

In another embodiment, the invention provides a cell comprising one of the dsRNAs of the invention. The cell is generally a mammalian cell, such as a human cell.

In another embodiment, the invention provides a pharmaceutical composition for inhibiting the expression of the Eg5 gene in an organism, generally a human

subject, comprising one or more of the dsRNA of the invention and a pharmaceutically acceptable carrier or delivery vehicle.

In another embodiment, the invention provides a method for inhibiting the expression of the Eg5 gene in a cell, comprising the following steps:

(a) introducing into the cell a double-stranded ribonucleic acid (dsRNA), wherein the dsRNA comprises at least two sequences that are complementary to each other. The dsRNA comprises a sense strand comprising a first sequence and an antisense strand comprising a second sequence. The antisense strand comprises a region of complementarity which is substantially complementary to at least a part of a mRNA encoding Eg5, and wherein the region of complementarity is less than 30 nucleotides in length, generally 19-24 nucleotides in length, and wherein the dsRNA, upon contact with a cell expressing the Eg5, inhibits expression of the Eg5 gene by at least 40%; and

(b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of the Eg5 gene, thereby inhibiting expression of the Eg5 gene in the cell.

In another embodiment, the invention provides methods for treating, preventing or managing pathological processes mediated by Eg5 expression, e.g. cancer, comprising administering to a patient in need of such treatment, prevention or management a therapeutically or prophylactically effective amount of one or more of the dsRNAs of the invention.

In another embodiment, the invention provides vectors for inhibiting the expression of the Eg5 gene in a cell, comprising a regulatory sequence operably linked

to a nucleotide sequence that encodes at least one strand of one of the dsRNA of the invention.

In another embodiment, the invention provides a cell comprising a vector for inhibiting the expression of the Eg5 gene in a cell. The vector comprises a regulatory sequence operably linked to a nucleotide sequence that encodes at least one strand of one of the dsRNA of the invention.

In a further embodiment, the invention provides the Eg5 dsRNA and the uses thereof as described above in combination with a second dsRNA targeting the VEGF mRNA. A combination of a dsRNA targeting Eg5 and a second dsRNA targeting VEGF provides complementary and synergiatic activity for treating hyperproliferative discords, particulary hepatic carcinoma.

Brief Description of the Figures

No Figures are presented

Detailed Description of the Invention

The invention provides double-stranded ribonucleic acid (dsRNA), as well as compositions and methods for inhibiting the expression of the Eg5 gene in a cell or mammal using the dsRNA. The invention also provides compositions and methods for treating pathological conditions and diseases in a mammal caused by the expression of the Eg5 gene using dsRNA. dsRNA directs the sequence-specific degradation of mRNA through a process known as RNA interference (RNAi). The invention further provides this dsRNA in combination with a second dsRNA that inhibits the expression of the VEGF gene.

The dsRNAs of the invention comprises an RNA strand (the antisense strand) having a region which is less than 30 nucleotides in length, generally 19-24 nucleotides in length, and is substantially complementary to at least part of an mRNA

transcript of the Eg5 gene. The use of these dsRNAs enables the targeted degradation of mRNAs of genes that are implicated in replication and or maintenance of cancer cells in mammals. Using cell-based and animal assays, the present inventors have demonstrated that very low dosages of these dsRNA can specifically and efficiently mediate RNAi, resulting in significant inhibition of expression of the Eg5 gene. Thus, the methods and compositions of the invention comprising these dsRNAs are useful for treating pathological processes mediated by Eg5 expression, e.g. cancer, by targeting a gene involved in mitotic division.

The following detailed description discloses how to make and use the dsRNA and compositions containing dsRNA to inhibit the expression of the Eg5 gene, as well as compositions and methods for treating diseases and disorders caused by the expression of Eg5, such as cancer, alone or in combination with a second dsRNA targeting the VEGF gene. The pharmaceutical compositions of the invention comprise a dsRNA having an antisense strand comprising a region of complementarity which is less than 30 nucleotides in length, generally 19-24 nucleotides in length, and is substantially complementary to at least part of an RNA transcript of the Eg5 gene, together with a pharmaceutically acceptable carrier. As discussed above, such compositions can further include a second dsRNA targeting VEGF.

Accordingly, certain aspects of the invention provide pharmaceutical compositions comprising the dsRNA of the invention together with a pharmaceutically acceptable carrier, methods of using the compositions to inhibit expression of the Eg5 gene, and methods of using the pharmaceutical compositions to treat diseases caused by expression of the Eg5 gene. The invention further provides the above pharmaceutical compositions further containing a second dsRNA designed to inhibit the expression of VEGF.

I. Definitions

For convenience, the meaning of certain terms and phrases used in the specification, examples, and appended claims, are provided below. If there is an apparent discrepancy between the usage of a term in other parts of this specification and its definition provided in this section, the definition in this section shall prevail.

"G," "C," "A" and "U" each generally stand for a nucleotide that contains guanine, cytosine, adenine, and uracil as a base, respectively. However, it will be understood that the term "ribonucleotide" or "nucleotide" can also refer to a modified nucleotide, as further detailed below, or a surrogate replacement moiety. The skilled person is well aware that guanine, cytosine, adenine, and uracil may be replaced by other moieties without substantially altering the base pairing properties of an oligonucleotide comprising a nucleotide bearing such replacement moiety. For example, without limitation, a nucleotide comprising inosine as its base may base pair with nucleotides containing adenine, cytosine, or uracil. Hence, nucleotides containing uracil, guanine, or adenine may be replaced in the nucleotide sequences of the invention by a nucleotide containing, for example, inosine. Sequences comprising such replacement moieties are embodiments of the invention.

As used herein, "Eg5" refers to the human kinesin family member 11, which is also known as KIF11, Eg5, HKSP, KNSL1 or TRIP5. Eg5 sequence can be found as NCBI GeneID:3832, HGNC ID: HGNC:6388 and RefSeq ID number:NM_004523.

As used herein, "target sequence" refers to a contiguous portion of the nucleotide sequence of an mRNA molecule formed during the transcription of the Eg5 gene, including mRNA that is a product of RNA processing of a primary transcription product.

As used hereing, VEGF, also known as vascular permeability factor, is an angiogenic growth factor. VEGF is a homodimeric 45 kDa glycoprotein that exists in at least three different isoforms. VEGF isoforms are expressed in endothelial cells. The VEGF gene contains 8 exons that express a 189-amino acid protein isoform. A 165-amino acid isoform lacks the residues encoded by exon 6, whereas a 121-amino acid isoform lacks the residues encoded by exons 6 and 7. VEGF145 is an isoform predicted to contain 145 amino acids and to lack exon 7. VEGF can act on endothelial cells by binding to an endothelial tyrosine kinase receptor, such as Flt-1 (VEGFR-1) or KDR/flk-1 (VEGFR-2). VEGFR-2 is expressed in endothelial cells and is involved in endothelial cell differentiation and vasculogenesis. A third receptor, VEGFR-3 has been implicated in lymphogenesis.

The various isoforms have different biologic activities and clinical implications. For example, VEGF145 induces angiogenesis and like VEGF189 (but unlike VEGF165) VEGF145 binds efficiently to the extracellular matrix by a mechanism that is not dependent on extracellular matrix-associated heparin sulfates. VEGF displays activity as an endothelial cell mitogen and chemoattractant *in vitro* and induces vascular permeability and angiogenesis *in vivo*. VEGF is secreted by a wide variety of cancer cell types and promotes the growth of tumors by inducing the development of tumor-associated vasculature. Inhibition of VEGF function has been shown to limit both the growth of primary experimental tumors as well as the incidence of metastases in immunocompromised mice. Various dsRNAs directed to VEGF are described in co-pending US Ser. No. 11/078,073 ans 11/340,080, herein incorporated by reference).

As used herein, the term "strand comprising a sequence" refers to an oligonucleotide comprising a chain of nucleotides that is described by the sequence referred to using the standard nucleotide nomenclature.

As used herein, and unless otherwise indicated, the term "complementary," when used to describe a first nucleotide sequence in relation to a second nucleotide sequence, refers to the ability of an oligonucleotide or polynucleotide comprising the first nucleotide sequence to hybridize and form a duplex structure under certain conditions with an oligonucleotide or polynucleotide comprising the second nucleotide sequence, as will be understood by the skilled person. Such conditions can, for example, be stringent conditions, where stringent conditions may include: 400 mM NaCl, 40 mM PIPES pH 6.4, 1 mM EDTA, 50°C or 70°C for 12-16 hours followed by washing. Other conditions, such as physiologically relevant conditions as may be encountered inside an organism, can apply. The skilled person will be able to determine the set of conditions most appropriate for a test of complementarity of two sequences in accordance with the ultimate application of the hybridized nucleotides.

This includes base-pairing of the oligonucleotide or polynucleotide comprising the first nucleotide sequence to the oligonucleotide or polynucleotide comprising the second nucleotide sequence over the entire length of the first and second nucleotide sequence. Such sequences can be referred to as "fully complementary" with respect to each other herein. However, where a first sequence is referred to as "substantially complementary" with respect to a second sequence herein, the two sequences can be fully complementary, or they may form one or more, but generally not more than 4, 3 or 2 mismatched base pairs upon hybridization, while retaining the ability to hybridize under the conditions most relevant to their ultimate application. However, where two oligonucleotides are designed to form, upon hybridization, one or more single stranded overhangs, such overhangs shall not be regarded as mismatches with regard to the determination of complementarity. For example, a dsRNA comprising one oligonucleotide 21 nucleotides in length and another oligonucleotide 23 nucleotides in length, wherein the longer oligonucleotide comprises a sequence of 21 nucleotides that is fully complementary to the shorter oligonucleotide, may yet be referred to as "fully complementary" for the purposes of the invention.

"Complementary" sequences, as used herein, may also include, or be formed entirely from, non-Watson-Crick base pairs and/or base pairs formed from non-natural and modified nucleotides, in as far as the above requirements with respect to their ability to hybridize are fulfilled.

The terms "complementary", "fully complementary" and "substantially complementary" herein may be used with respect to the base matching between the sense strand and the antisense strand of a dsRNA, or between the antisense strand of a dsRNA and a target sequence, as will be understood from the context of their use.

As used herein, a polynucleotide which is "substantially complementary to at least part of" a messenger RNA (mRNA) refers to a polynucleotide which is substantially complementary to a contiguous portion of the mRNA of interest (e.g., encoding Eg5). For example, a polynucleotide is complementary to at least a part of a Eg5 mRNA if the sequence is substantially complementary to a non-interrupted portion of a mRNA encoding Eg5.

The term "double-stranded RNA" or "dsRNA", as used herein, refers to a complex of ribonucleic acid molecules, having a duplex structure comprising two anti-parallel and substantially complementary, as defined above, nucleic acid strands,. The two strands forming the duplex structure may be different portions of one larger RNA molecule, or they may be separate RNA molecules. Where the two strands are part of one larger molecule, and therefore are connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'end of the respective other strand forming the duplex structure, the connecting RNA chain is referred to as a "hairpin loop". Where the two strands are connected covalently by means other than an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'end of the respective other strand forming the duplex structure, the connecting structure is referred to as a "linker". The RNA strands may have the same or a different number of nucleotides. The maximum number of base pairs is the number of nucleotides in

the shortest strand of the dsRNA minus any overhangs that are present in the duplex. In addition to the duplex structure, a dsRNA may comprise one or more nucleotide overhangs.

As used herein, a "nucleotide overhang" refers to the unpaired nucleotide or nucleotides that protrude from the duplex structure of a dsRNA when a 3'-end of one strand of the dsRNA extends beyond the 5'-end of the other strand, or vice versa. "Blunt" or "blunt end" means that there are no unpaired nucleotides at that end of the dsRNA, i.e., no nucleotide overhang. A "blunt ended" dsRNA is a dsRNA that is double-stranded over its entire length, i.e., no nucleotide overhang at either end of the molecule.

The term "antisense strand" refers to the strand of a dsRNA which includes a region that is substantially complementary to a target sequence. As used herein, the term "region of complementarity" refers to the region on the antisense strand that is substantially complementary to a sequence, for example a target sequence, as defined herein. Where the region of complementarity is not fully complementary to the target sequence, the mismatches are most tolerated in the terminal regions and, if present, are generally in a terminal region or regions, *e.g.*, within 6, 5, 4, 3, or 2 nucleotides of the 5' and/or 3' terminus.

The term "sense strand," as used herein, refers to the strand of a dsRNA that includes a region that is substantially complementary to a region of the antisense strand.

"Introducing into a cell", when referring to a dsRNA, means facilitating uptake or absorption into the cell, as is understood by those skilled in the art. Absorption or uptake of dsRNA can occur through unaided diffusive or active cellular processes, or by auxiliary agents or devices. The meaning of this term is not limited to cells in vitro; a dsRNA may also be "introduced into a cell", wherein the cell is part of a living

organism. In such instance, introduction into the cell will include the delivery to the organism. For example, for in vivo delivery, dsRNA can be injected into a tissue site or administered systemically. In vitro introduction into a cell includes methods known in the art such as electroporation and lipofection.

The terms "silence" and "inhibit the expression of", in as far as they refer to the Eg5 gene, herein refer to the at least partial suppression of the expression of the Eg5 gene, as manifested by a reduction of the amount of mRNA transcribed from the Eg5 gene which may be isolated from a first cell or group of cells in which the Eg5 gene is transcribed and which has or have been treated such that the expression of the Eg5 gene is inhibited, as compared to a second cell or group of cells substantially identical to the first cell or group of cells but which has or have not been so treated (control cells). The degree of inhibition is usually expressed in terms of

$$\frac{(\text{mRNA in control cells}) - (\text{mRNA in treated cells})}{(\text{mRNA in control cells})} * 100\%$$

Alternatively, the degree of inhibition may be given in terms of a reduction of a parameter that is functionally linked to Eg5 gene transcription, e.g. the amount of protein encoded by the Eg5 gene which is secreted by a cell, or the number of cells displaying a certain phenotype, e.g apoptosis. In principle, Eg5 gene silencing may be determined in any cell expressing the target, either constitutively or by genomic engineering, and by any appropriate assay. However, when a reference is needed in order to determine whether a given dsRNA inhibits the expression of the Eg5 gene by a certain degree and therefore is encompassed by the instant invention, the assay provided in the Examples below shall serve as such reference.

For example, in certain instances, expression of the Eg5 gene (or VEGF gene) is suppressed by at least about 20%, 25%, 35%, or 50% by administration of the double-stranded oligonucleotide of the invention. In some embodiment, the Eg5 gene

is suppressed by at least about 60%, 70%, or 80% by administration of the double-stranded oligonucleotide of the invention. In some embodiments, the Eg5 gene is suppressed by at least about 85%, 90%, or 95% by administration of the double-stranded oligonucleotide of the invention. Tables 1-3 provides values for inhibition of expression using various Eg5 dsRNA molecules at various concentrations.

As used herein in the context of Eg5 expression, the terms "treat", "treatment", and the like, refer to relief from or alleviation of pathological processes mediated by Eg5 expression. In the context of the present invention insofar as it relates to any of the other conditions recited herein below (other than pathological processes mediated by Eg5 expression), the terms "treat", "treatment", and the like mean to relieve or alleviate at least one symptom associated with such condition, or to slow or reverse the progression of such condition, such as the slowing and progression of hepatic carcinoma.

As used herein, the phrases "therapeutically effective amount" and "prophylactically effective amount" refer to an amount that provides a therapeutic benefit in the treatment, prevention, or management of pathological processes mediated by Eg5 expression or an overt symptom of pathological processes mediated by Eg5 expression (alone or in combination with VEGF expression). The specific amount that is therapeutically effective can be readily determined by ordinary medical practitioner, and may vary depending on factors known in the art, such as, e.g. the type of pathological processes mediated by Eg5 expression, the patient's history and age, the stage of pathological processes mediated by Eg5 expression, and the administration of other anti-pathological processes mediated by Eg5 expression agents.

As used herein, a "pharmaceutical composition" comprises a pharmacologically effective amount of a dsRNA and a pharmaceutically acceptable carrier. As used herein, "pharmacologically effective amount," "therapeutically

effective amount" or simply "effective amount" refers to that amount of an RNA effective to produce the intended pharmacological, therapeutic or preventive result. For example, if a given clinical treatment is considered effective when there is at least a 25% reduction in a measurable parameter associated with a disease or disorder, a therapeutically effective amount of a drug for the treatment of that disease or disorder is the amount necessary to effect at least a 25% reduction in that parameter.

The term "pharmaceutically acceptable carrier" refers to a carrier for administration of a therapeutic agent. Such carriers include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The term specifically excludes cell culture medium. For drugs administered orally, pharmaceutically acceptable carriers include, but are not limited to pharmaceutically acceptable excipients such as inert diluents, disintegrating agents, binding agents, lubricating agents, sweetening agents, flavoring agents, coloring agents and preservatives. Suitable inert diluents include sodium and calcium carbonate, sodium and calcium phosphate, and lactose, while corn starch and alginic acid are suitable disintegrating agents. Binding agents may include starch and gelatin, while the lubricating agent, if present, will generally be magnesium stearate, stearic acid or talc. If desired, the tablets may be coated with a material such as glyceryl monostearate or glyceryl distearate, to delay absorption in the gastrointestinal tract.

As used herein, a "transformed cell" is a cell into which a vector has been introduced from which a dsRNA molecule may be expressed.

II. Double-stranded ribonucleic acid (dsRNA)

In one embodiment, the invention provides double-stranded ribonucleic acid (dsRNA) molecules for inhibiting the expression of the Eg5 gene (alone or incombinaton with a second dsRNA for inhibiting the expression of VEGF) in a cell or mammal, wherein the dsRNA comprises an antisense strand comprising a region of complementarity which is complementary to at least a part of an mRNA formed in the

expression of the Eg5 gene, and wherein the region of complementarity is less than 30 nucleotides in length, generally 19-24 nucleotides in length, and wherein said dsRNA, upon contact with a cell expressing said Eg5 gene, inhibits the expression of said Eg5 gene by at least 40%. The dsRNA comprises two RNA strands that are sufficiently complementary to hybridize to form a duplex structure. One strand of the dsRNA (the antisense strand) comprises a region of complementarity that is substantially complementary, and generally fully complementary, to a target sequence, derived from the sequence of an mRNA formed during the expression of the Eg5 gene, the other strand (the sense strand) comprises a region which is complementary to the antisense strand, such that the two strands hybridize and form a duplex structure when combined under suitable conditions. Generally, the duplex structure is between 15 and 30, more generally between 18 and 25, yet more generally between 19 and 24, and most generally between 19 and 21 base pairs in length. Similarly, the region of complementarity to the target sequence is between 15 and 30, more generally between 18 and 25, yet more generally between 19 and 24, and most generally between 19 and 21 nucleotides in length. The dsRNA of the invention may further comprise one or more single-stranded nucleotide overhang(s). The dsRNA can be synthesized by standard methods known in the art as further discussed below, e.g., by use of an automated DNA synthesizer, such as are commercially available from, for example, Biosearch, Applied Biosystems, Inc. In a preferred embodiment, the Eg5 gene is the human Eg5 gene. In specific embodiments, the antisense strand of the dsRNA comprises the sense sequences of Tables 1-3 and the second sequence is selected from the group consisting of the antisense sequences of Tables 1-3. Alternative antisense agents that target elsewhere in the target sequence provided in Tables 1-3 can readily be determined using the target sequence and the flanking Eg5 sequence. In embodiments using a second dsRNA targeting VEGF, such agents are exemplified in the Examples and in co-pending US Serial Nos: 11/078,073 and 11/340,080, herein incorporated by reference.

The dsRNA will comprise at least two nucleotide sequence selected from the groups of sequences provided in Tables 1-3. One of the two sequences is complementary to the other of the two sequences, with one of the sequences being substantially complementary to a sequence of an mRNA generated in the expression of the Eg5 gene. As such, the dsRNA will comprises two oligonucleotides, wherein one oligonucleotide is described as the sense strand in Tables 1-3 and the second oligonucleotide is described as the antisense strand in Tables 1-3

The skilled person is well aware that dsRNAs comprising a duplex structure of between 20 and 23, but specifically 21, base pairs have been hailed as particularly effective in inducing RNA interference (Elbashir et al., EMBO 2001, 20:6877-6888). However, others have found that shorter or longer dsRNAs can be effective as well. In the embodiments described above, by virtue of the nature of the oligonucleotide sequences provided in Tables 1-3, the dsRNAs of the invention can comprise at least one strand of a length of minimally 21 nt. It can be reasonably expected that shorter dsRNAs comprising one of the sequences of Tables 1-3 minus only a few nucleotides on one or both ends may be similarly effective as compared to the dsRNAs described above. Hence, dsRNAs comprising a partial sequence of at least 15, 16, 17, 18, 19, 20, or more contiguous nucleotides from one of the sequences of Tables 1-3, and differing in their ability to inhibit the expression of the Eg5 gene in a FACS assay as described herein below by not more than 5, 10, 15, 20, 25, or 30 % inhibition from a dsRNA comprising the full sequence, are contemplated by the invention. Further dsRNAs that cleave within the target sequence provided in Tables 1-3 can readily be made using the Eg5 sequence and the target sequence provided.

In addition, the RNAi agents provided in Tables 1-3 identify a site in the Eg5 mRNA that is susceptible to RNAi based cleavage. As such the present invention further includes RNAi agents that target within the sequence targeted by one of the agents of the present invention. As used herein a second RNAi agent is said to target within the sequence of a first RNAi agent if the second RNAi agent cleaves the

message anywhere within the mRNA that is complementary to the antisense strand of the first RNAi agent. Such a second agent will generally consist of at least 15 contiguous nucleotides from one of the sequences provided in Tables 1-3 coupled to additional nucleotide sequences taken from the region contiguous to the selected sequence in the Eg5 gene. For example, the last 15 nucleotides of SEQ ID NO:1 combined with the next 6 nucleotides from the target Eg5 gene produces a single strand agent of 21 nucleotides that is based on one of the sequences provided in Tables 1-3.

The dsRNA of the invention can contain one or more mismatches to the target sequence. In a preferred embodiment, the dsRNA of the invention contains no more than 3 mismatches. If the antisense strand of the dsRNA contains mismatches to a target sequence, it is preferable that the area of mismatch not be located in the center of the region of complementarity. If the antisense strand of the dsRNA contains mismatches to the target sequence, it is preferable that the mismatch be restricted to 5 nucleotides from either end, for example 5, 4, 3, 2, or 1 nucleotide from either the 5' or 3' end of the region of complementarity. For example, for a 23 nucleotide dsRNA strand which is complementary to a region of the Eg5 gene, the dsRNA generally does not contain any mismatch within the central 13 nucleotides. The methods described within the invention can be used to determine whether a dsRNA containing a mismatch to a target sequence is effective in inhibiting the expression of the Eg5 gene. Consideration of the efficacy of dsRNAs with mismatches in inhibiting expression of the Eg5 gene is important, especially if the particular region of complementarity in the Eg5 gene is known to have polymorphic sequence variation within the population.

In one embodiment, at least one end of the dsRNA has a single-stranded nucleotide overhang of 1 to 4, generally 1 or 2 nucleotides. dsRNAs having at least one nucleotide overhang have unexpectedly superior inhibitory properties than their blunt-ended counterparts. Moreover, the present inventors have discovered that the presence of only one nucleotide overhang strengthens the interference activity of the

dsRNA, without affecting its overall stability. dsRNA having only one overhang has proven particularly stable and effective in vivo, as well as in a variety of cells, cell culture mediums, blood, and serum. Generally, the single-stranded overhang is located at the 3'-terminal end of the antisense strand or, alternatively, at the 3'-terminal end of the sense strand. The dsRNA may also have a blunt end, generally located at the 5'-end of the antisense strand. Such dsRNAs have improved stability and inhibitory activity, thus allowing administration at low dosages, i.e., less than 5 mg/kg body weight of the recipient per day. Generally, the antisense strand of the dsRNA has a nucleotide overhang at the 3'-end, and the 5'-end is blunt. In another embodiment, one or more of the nucleotides in the overhang is replaced with a nucleoside thiophosphate.

In yet another embodiment, the dsRNA is chemically modified to enhance stability. The nucleic acids of the invention may be synthesized and/or modified by methods well established in the art, such as those described in "Current protocols in nucleic acid chemistry", Beaucage, S.L. et al. (Edrs.), John Wiley & Sons, Inc., New York, NY, USA, which is hereby incorporated herein by reference. Specific examples of preferred dsRNA compounds useful in this invention include dsRNAs containing modified backbones or no natural internucleoside linkages. As defined in this specification, dsRNAs having modified backbones include those that retain a phosphorus atom in the backbone and those that do not have a phosphorus atom in the backbone. For the purposes of this specification, and as sometimes referenced in the art, modified dsRNAs that do not have a phosphorus atom in their internucleoside backbone can also be considered to be oligonucleosides.

Preferred modified dsRNA backbones include, for example, phosphorothioates, chiral phosphorothioates, phosphorodithioates, phosphotriesters, aminoalkylphosphotriesters, methyl and other alkyl phosphonates including 3'-alkylene phosphonates and chiral phosphonates, phosphinates, phosphoramidates

including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates having normal 3'-5' linkages, 2'-5' linked analogs of these, and those) having inverted polarity wherein the adjacent pairs of nucleoside units are linked 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included.

Representative U.S. patents that teach the preparation of the above phosphorus-containing linkages include, but are not limited to, U.S. Pat. Nos. 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,195; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,316; 5,550,111; 5,563,253; 5,571,799; 5,587,361; and 5,625,050, each of which is herein incorporated by reference

Preferred modified dsRNA backbones that do not include a phosphorus atom therein have backbones that are formed by short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatoms and alkyl or cycloalkyl internucleoside linkages, or ore or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH2 component parts.

Representative U.S. patents that teach the preparation of the above oligonucleosides include, but are not limited to, U.S. Pat. Nos. 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,64,562; 5,264,564; 5,405,938;

5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and, 5,677,439, each of which is herein incorporated by reference.

In other preferred dsRNA mimetics, both the sugar and the internucleoside linkage, i.e., the backbone, of the nucleotide units are replaced with novel groups. The base units are maintained for hybridization with an appropriate nucleic acid target compound. One such oligomeric compound, an dsRNA mimetic that has been shown to have excellent hybridization properties, is referred to as a peptide nucleic acid (PNA). In PNA compounds, the sugar backbone of an dsRNA is replaced with an amide containing backbone, in particular an aminoethylglycine backbone. The nucleobases are retained and are bound directly or indirectly to aza nitrogen atoms of the amide portion of the backbone. Representative U.S. patents that teach the preparation of PNA compounds include, but are not limited to, U.S. Pat. Nos. 5,539,082; 5,714,331; and 5,719,262, each of which is herein incorporated by reference. Further teaching of PNA compounds can be found in Nielsen et al., Science, 1991, 254, 1497-1500.

Most preferred embodiments of the invention are dsRNAs with phosphorothioate backbones and oligonucleosides with heteroatom backbones, and in particular --CH.sub.2--NH--CH.sub.2--, --CH.sub.2--N(CH.sub.3)--O--CH.sub.2-- [known as a methylene (methylimino) or MMI backbone], --CH.sub.2--O--N(CH.sub.3)--CH.sub.2--, --CH.sub.2--N(CH.sub.3)--N(CH.sub.3)--CH.sub.2-- and --N(CH.sub.3)--CH.sub.2--CH.sub.2--[wherein the native phosphodiester backbone is represented as --O--P--O--CH.sub.2--] of the above-referenced U.S. Pat. No. 5,489,677, and the amide backbones of the above-referenced U.S. Pat. No. 5,602,240. Also preferred are dsRNAs having morpholino backbone structures of the above-referenced U.S. Pat. No. 5,034,506.

Modified dsRNAs may also contain one or more substituted sugar moieties. Preferred dsRNAs comprise one of the following at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl may be substituted or unsubstituted C.sub.1 to C.sub.10 alkyl or C.sub.2 to C.sub.10 alkenyl and alkynyl. Particularly preferred are O[(CH.sub.2).sub.nO].sub.mCH.sub.3, O(CH.sub.2).sub.nOCH.sub.3, O(CH.sub.2).sub.nNH.sub.2, O(CH.sub.2).sub.nCH.sub.3, O(CH.sub.2).sub.nONH.sub.2, and O(CH.sub.2).sub.nON[(CH.sub.2).sub.nCH.sub.3)].sub.2, where n and m are from 1 to about 10. Other preferred dsRNAs comprise one of the following at the 2' position: C.sub.1 to C.sub.10 lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH.sub.3, OCN, Cl, Br, CN, CF.sub.3, OCF.sub.3, SOCH.sub.3, SO.sub.2CH.sub.3, ONO.sub.2, NO.sub.2, N.sub.3, NH.sub.2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an dsRNA, or a group for improving the pharmacodynamic properties of an dsRNA, and other substituents having similar properties. A preferred modification includes 2'-methoxyethoxy (2'-O--CH.sub.2CH.sub.2OCH.sub.3, also known as 2'-O-(2-methoxyethyl) or 2'-MOE) (Martin et al., Helv. Chim. Acta, 1995, 78, 486-504) i.e., an alkoxy-alkoxy group. A further preferred modification includes 2'-dimethylaminooxyethoxy, i.e., a O(CH.sub.2).sub.2ON(CH.sub.3).sub.2 group, also known as 2'-DMAOE, as described in examples hereinbelow, and 2'-dimethylaminoethoxyethoxy (also known in the art as 2'-O-dimethylaminoethoxyethyl or 2'-DMAEOE), i.e., 2'-O--CH.sub.2--O--CH.sub.2--N(CH.sub.2).sub.2, also described in examples hereinbelow.

Other preferred modifications include 2'-methoxy (2'-OCH.sub.3), 2'-aminopropoxy (2'-OCH.sub.2CH.sub.2CH.sub.2NH.sub.2) and 2'-fluoro (2'-F). Similar modifications may also be made at other positions on the dsRNA, particularly

the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked dsRNAs and the 5' position of 5' terminal nucleotide. DsRNAs may also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. Representative U.S. patents that teach the preparation of such modified sugar structures include, but are not limited to, U.S. Pat. Nos. 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, certain of which are commonly owned with the instant application, and each of which is herein incorporated by reference in its entirety.

DsRNAs may also include nucleobase (often referred to in the art simply as "base") modifications or substitutions. As used herein, "unmodified" or "natural" nucleobases include the purine bases adenine (A) and guanine (G), and the pyrimidine bases thymine (T), cytosine (C) and uracil (U). Modified nucleobases include other synthetic and natural nucleobases such as 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl anal other 8-substituted adenines and guanines, 5-halo, particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-daazaadenine and 3-deazaguanine and 3-deazaadenine. Further nucleobases include those disclosed in U.S. Pat. No. 3,687,808, those disclosed in The Concise Encyclopedia Of Polymer Science And Engineering, pages 858-859, Kroschwitz, J. L, ed. John Wiley & Sons, 1990, these disclosed by Englisch et al., Angewandte Chemie, International Edition, 1991, 30, 613, and those disclosed by Sanghvi, Y S., Chapter 15, DsRNA Research and Applications, pages 289-302, Crooke, S. T. and Lebleu, B., Ed., CRC Press, 1993.

Certain of these nucleobases are particularly useful for increasing the binding affinity of the oligomeric compounds of the invention. These include 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and 0-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine substitutions have been shown to increase nucleic acid duplex stability by 0.6-1.2.degree. C. (Sanghvi, Y. S., Crooke, S. T. and Lebleu, B., Eds., DsRNA Research and Applications, CRC Press, Boca Raton, 1993, pp. 276-278) and are presently preferred base substitutions, even more particularly when combined with 2'-O-methoxyethyl sugar modifications.

Representative U.S. patents that teach the preparation of certain of the above noted modified nucleobases as well as other modified nucleobases include, but are not limited to, the above noted U.S. Pat. No. 3,687,808, as well as U.S. Pat. Nos. 4,845,205; 5,130,30; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; and 5,681,941, each of which is herein incorporated by reference, and U.S. Pat. No. 5,750,692, also herein incorporated by reference.

Another modification of the dsRNAs of the invention involves chemically linking to the dsRNA one or more moieties or conjugates which enhance the activity, cellular distribution or cellular uptake of the dsRNA. Such moieties include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger et al., Proc. Natl. Acid. Sci. USA, 199, 86, 6553-6556), cholic acid (Manoharan et al., Biorg. Med. Chem. Let., 1994 4 1053-1060), a thioether, e.g., beryl-S-tritylthiol (Manoharan et al., Ann. N.Y. Acad. Sci., 1992, 660, 306-309; Manoharan et al., Biorg. Med. Chem. Let., 1993, 3, 2765-2770), a thiocholesterol (Oberhauser et al., Nucl. Acids Res., 1992, 20, 533-538), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., EMBO J, 1991, 10, 1111-1118; Kabanov et al., FEBS Lett., 1990, 259, 327-330; Svinarchuk et al., Biochimie, 1993, 75, 49-54), a phospholipid, e.g., di-

hexadecyl-rac-glycerol or triethyl-ammonium 1,2-di-O-hexadecyl-rac-glycero-3-Hphosphonate (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651-3654; Shea et al., Nucl. Acids Res., 1990, 18, 3777-3783), a polyamine or a polyethylene glycol chain (Manoharan et al., Nucleosides & Nucleotides, 1995, 14, 969-973), or adamantane acetic acid (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651-3654), a palmityl moiety (Mishra et al., Biochim. Biophys. Acta, 1995, 1264, 229-237), or an octadecylamine or hexylamino-carbonyloxycholesterol moiety (Crooke et al., J. Pharmacol. Exp. Ther., 1996, 277, 923-937).

Representative U.S. patents that teach the preparation of such dsRNA conjugates include, but are not limited to, U.S. Pat. Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717, 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391,723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941, each of which is herein incorporated by reference.

It is not necessary for all positions in a given compound to be uniformly modified, and in fact more than one of the aforementioned modifications may be incorporated in a single compound or even at a single nucleoside within an dsRNA. The present invention also includes dsRNA compounds which are chimeric compounds. "Chimeric" dsRNA compounds or "chimeras," in the context of this invention, are dsRNA compounds, particularly dsRNAs, which contain two or more chemically distinct regions, each made up of at least one monomer unit, i.e., a nucleotide in the case of an dsRNA compound. These dsRNAs typically contain at

least one region wherein the dsRNA is modified so as to confer upon the dsRNA increased resistance to nuclease degradation, increased cellular uptake, and/or increased binding affinity for the target nucleic acid. An additional region of the dsRNA may serve as a substrate for enzymes capable of cleaving RNA:DNA or RNA:RNA hybrids. By way of example, RNase H is a cellular endonuclease which cleaves the RNA strand of an RNA:DNAduplex. Activation of RNase H, therefore, results in cleavage of the RNA target, thereby greatly enhancing the efficiency of dsRNA inhibition of gene expression. Consequently, comparable results can often be obtained with shorter dsRNAs when chimeric dsRNAs are used, compared to phosphorothioate deoxydsRNAs hybridizing to the same target region. Cleavage of the RNA target can be routinely detected by gel electrophoresis and, if necessary, associated nucleic acid hybridization techniques known in the art.

In certain instances, the dsRNA may be modified by a non-ligand group. A number of non-ligand molecules have been conjugated to dsRNAs in order to enhance the activity, cellular distribution or cellular uptake of the dsRNA, and procedures for performing such conjugations are available in the scientific literature. Such non-ligand moieties have included lipid moieties, such as cholesterol (Letsinger et al., Proc. Natl. Acad. Sci. USA, 1989, 86:6553), cholic acid (Manoharan et al., Bioorg. Med. Chem. Lett., 1994, 4:1053), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., Ann. N.Y. Acad. Sci., 1992, 660:306; Manoharan et al., Bioorg. Med. Chem. Let., 1993, 3:2765), a thiocholesterol (Oberhauser et al., Nucl. Acids Res., 1992, 20:533), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., EMBO J., 1991, 10:111; Kabanov et al., FEBS Lett., 1990, 259:327; Svinarchuk et al., Biochimie, 1993, 75:49), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., Tetrahedron Lett., 1995, 36:3651; Shea et al., Nucl. Acids Res., 1990, 18:3777), a polyamine or a polyethylene glycol chain (Manoharan et al., Nucleosides & Nucleotides, 1995, 14:969), or adamantane acetic acid (Manoharan et al., Tetrahedron

Lett., 1995, 36:3651), a palmityl moiety (Mishra et al., Biochim. Biophys. Acta, 1995, 1264:229), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., J. Pharmacol. Exp. Ther., 1996, 277:923). Representative United States patents that teach the preparation of such dsRNA conjugates have been listed above. Typical conjugation protocols involve the synthesis of dsRNAs bearing an aminolinker at one or more positions of the sequence. The amino group is then reacted with the molecule being conjugated using appropriate coupling or activating reagents. The conjugation reaction may be performed either with the dsRNA still bound to the solid support or following cleavage of the dsRNA in solution phase. Purification of the dsRNA conjugate by HPLC typically affords the pure conjugate.

Vector encoded RNAi agents

The dsRNA of the invention can also be expressed from recombinant viral vectors intracellularly in vivo. The recombinant viral vectors of the invention comprise sequences encoding the dsRNA of the invention and any suitable promoter for expressing the dsRNA sequences. Suitable promoters include, for example, the U6 or H1 RNA pol III promoter sequences and the cytomegalovirus promoter. Selection of other suitable promoters is within the skill in the art. The recombinant viral vectors of the invention can also comprise inducible or regulatable promoters for expression of the dsRNA in a particular tissue or in a particular intracellular environment. The use of recombinant viral vectors to deliver dsRNA of the invention to cells in vivo is discussed in more detail below.

dsRNA of the invention can be expressed from a recombinant viral vector either as two separate, complementary RNA molecules, or as a single RNA molecule with two complementary regions.

Any viral vector capable of accepting the coding sequences for the dsRNA molecule(s) to be expressed can be used, for example vectors derived from adenovirus

(AV); adeno-associated virus (AAV); retroviruses (e.g, lentiviruses (LV), Rhabdoviruses, murine leukemia virus); herpes virus, and the like. The tropism of viral vectors can be modified by pseudotyping the vectors with envelope proteins or other surface antigens from other viruses, or by substituting different viral capsid proteins, as appropriate.

For example, lentiviral vectors of the invention can be pseudotyped with surface proteins from vesicular stomatitis virus (VSV), rabies, Ebola, Mokola, and the like. AAV vectors of the invention can be made to target different cells by engineering the vectors to express different capsid protein serotypes. For example, an AAV vector expressing a serotype 2 capsid on a serotype 2 genome is called AAV 2/2. This serotype 2 capsid gene in the AAV 2/2 vector can be replaced by a serotype 5 capsid gene to produce an AAV 2/5 vector. Techniques for constructing AAV vectors which express different capsid protein serotypes are within the skill in the art; see, e.g., Rabinowitz J E et al. (2002), J Virol 76:791-801, the entire disclosure of which is herein incorporated by reference.

Selection of recombinant viral vectors suitable for use in the invention, methods for inserting nucleic acid sequences for expressing the dsRNA into the vector, and methods of delivering the viral vector to the cells of interest are within the skill in the art. See, for example, Dornburg R (1995), Gene Therap. 2: 301-310; Eglitis M A (1988), Biotechniques 6: 608-614; Miller A D (1990), Hum Gene Therap. 1: 5-14; Anderson W F (1998), Nature 392: 25-30; and Rubinson D A et al., Nat. Genet. 33: 401-406, the entire disclosures of which are herein incorporated by reference.

Preferred viral vectors are those derived from AV and AAV. In a particularly preferred embodiment, the dsRNA of the invention is expressed as two separate, complementary single-stranded RNA molecules from a recombinant AAV vector

comprising, for example, either the U6 or H1 RNA promoters, or the cytomegalovirus (CMV) promoter.

A suitable AV vector for expressing the dsRNA of the invention, a method for constructing the recombinant AV vector, and a method for delivering the vector into target cells, are described in Xia H et al. (2002), Nat. Biotech. 20: 1006-1010.

Suitable AAV vectors for expressing the dsRNA of the invention, methods for constructing the recombinant AV vector, and methods for delivering the vectors into target cells are described in Samulski R et al. (1987), J. Virol. 61: 3096-3101; Fisher K J et al. (1996), J. Virol, 70: 520-532; Samulski R et al. (1989), J. Virol. 63: 3822-3826; U.S. Pat. No. 5,252,479; U.S. Pat. No. 5,139,941; International Patent Application No. WO 94/13788; and International Patent Application No. WO 93/24641, the entire disclosures of which are herein incorporated by reference.

III. Pharmaceutical compositions comprising dsRNA

In one embodiment, the invention provides pharmaceutical compositions comprising a dsRNA, as described herein, and a pharmaceutically acceptable carrier. The pharmaceutical composition comprising the dsRNA is useful for treating a disease or disorder associated with the expression or activity of the Eg5 gene, such as pathological processes mediated by Eg5 expression. Such pharmaceutical compositions are formulated based on the mode of delivery. One example is compositions that are formulated for systemic administration via parenteral delivery.

In another embodiment, such compositions will further comprise a second dsRNA that inhibits VEGF expression. dsRNA directed to VEGF are described in the Examples and in co-pending US Serial Nos: 11/078,073 and 11/340,080.

The pharmaceutical compositions of the invention are administered in dosages sufficient to inhibit expression of the Eg5 gene (and VEGF expression when a second

dsRNA is included). In general, a suitable dose of dsRNA will be in the range of 0.01 to 5.0 milligrams per kilogram body weight of the recipient per day, generally in the range of 1 microgram to 1 mg per kilogram body weight per day. The pharmaceutical composition may be administered once daily or the dsRNA may be administered as two, three, or more sub-doses at appropriate intervals throughout the day or even using continuous infusion or delivery through a controlled release formulation. In that case, the dsRNA contained in each sub-dose must be correspondingly smaller in order to achieve the total daily dosage. The dosage unit can also be compounded for delivery over several days, e.g., using a conventional sustained release formulation which provides sustained release of the dsRNA over a several day period. Sustained release formulations are well known in the art and are particularly useful for delivery of agents at a particular site, such as could be used with the agents of the present invention. In this embodiment, the dosage unit contains a corresponding multiple of the daily dose.

The skilled artisan will appreciate that certain factors may influence the dosage and timing required to effectively treat a subject, including but not limited to the severity of the disease or disorder, previous treatments, the general health and/or age of the subject, and other diseases present. Moreover, treatment of a subject with a therapeutically effective amount of a composition can include a single treatment or a series of treatments. Estimates of effective dosages and in vivo half-lives for the individual dsRNAs encompassed by the invention can be made using conventional methodologies or on the basis of in vivo testing using an appropriate animal model, as described elsewhere herein.

Advances in mouse genetics have generated a number of mouse models for the study of various human diseases, such as pathological processes mediated by Eg5 expression. Such models are used for in vivo testing of dsRNA, as well as for determining a therapeutically effective dose.

The present invention also includes pharmaceutical compositions and formulations which include the dsRNA compounds of the invention. The pharmaceutical compositions of the present invention may be administered in a number of ways depending upon whether local or systemic treatment is desired and upon the area to be treated. Administration may be topical, pulmonary, e.g., by inhalation or insufflation of powders or aerosols, including by nebulizer; intratracheal, intranasal, epidermal and transdermal), oral or parenteral. Parenteral administration includes intravenous, intraarterial, subcutaneous, intraperitoneal or intramuscular injection or infusion; or intracranial, e.g., intrathecal or intraventricular, administration.

Pharmaceutical compositions and formulations for topical administration may include transdermal patches, ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like may be necessary or desirable. Coated condoms, gloves and the like may also be useful. Preferred topical formulations include those in which the dsRNAs of the invention are in admixture with a topical delivery agent such as lipids, liposomes, fatty acids, fatty acid esters, steroids, chelating agents and surfactants. Preferred lipids and liposomes include neutral (e.g. dioleoylphosphatidyl DOPE ethanolamine, dimyristoylphosphatidyl choline DMPC, distearolyphosphatidyl choline) negative (e.g. dimyristoylphosphatidyl glycerol DMPG) and cationic (e.g. dioleoyltetramethylaminopropyl DOTAP and dioleoylphosphatidyl ethanolamine DOTMA). DsRNAs of the invention may be encapsulated within liposomes or may form complexes thereto, in particular to cationic liposomes. Alternatively, dsRNAs may be complexed to lipids, in particular to cationic lipids. Preferred fatty acids and esters include but are not limited arachidonic acid, oleic acid, eicosanoic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a C_{1-10} alkyl ester (e.g. isopropylmyristate IPM), monoglyceride, diglyceride or pharmaceutically

acceptable salt thereof. Topical formulations are described in detail in U.S. patent application Ser. No. 09/315,298 filed on May 20, 1999 which is incorporated herein by reference in its entirety.

Compositions and formulations for oral administration include powders or granules, microparticulates, nanoparticulates, suspensions or solutions in water or non-aqueous media, capsules, gel capsules, sachets, tablets or minitablets. Thickeners, flavoring agents, diluents, emulsifiers, dispersing aids or binders may be desirable. Preferred oral formulations are those in which dsRNAs of the invention are administered in conjunction with one or more penetration enhancers surfactants and chelators. Preferred surfactants include fatty acids and/or esters or salts thereof, bile acids and/or salts thereof. Preferred bile acids/salts include chenodeoxycholic acid (CDCA) and ursodeoxychenodeoxycholic acid (UDCA), cholic acid, dehydrocholic acid, deoxycholic acid, glucholic acid, glycholic acid, glycodeoxycholic acid, taurocholic acid, taurodeoxycholic acid, sodium tauro-24,25-dihydro-fusidate and sodium glycodihydrofusidate. Preferred fatty acids include arachidonic acid, undecanoic acid, oleic acid, lauric acid, caprylic acid, capric acid, myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein, dilaurin, glyceryl 1-monocaprate, 1-dodecylazacycloheptan-2-one, an acylcarnitine, an acylcholine, or a monoglyceride, a diglyceride or a pharmaceutically acceptable salt thereof (e.g. sodium). Also preferred are combinations of penetration enhancers, for example, fatty acids/salts in combination with bile acids/salts. A particularly preferred combination is the sodium salt of lauric acid, capric acid and UDCA. Further penetration enhancers include polyoxyethylene-9-lauryl ether, polyoxyethylene-20-cetyl ether. DsRNAs of the invention may be delivered orally, in granular form including sprayed dried particles, or complexed to form micro or nanoparticles. DsRNA complexing agents include poly-amino acids; polyimines; polyacrylates; polyalkylacrylates, polyoxethanes, polyalkylcyanoacrylates; cationized gelatins, albumins, starches, acrylates, polyethyleneglycols (PEG) and starches; polyalkylcyanoacrylates; DEAE-derivatized polyimines, pollulans, celluloses and

starches. Particularly preferred complexing agents include chitosan, N-trimethylchitosan, poly-L-lysine, polyhistidine, polyornithine, polyspermines, protamine, polyvinylpyridine, polythiodiethylaminomethylethylene P(TDAE), polyaminostyrene (e.g. p-amino), poly(methylcyanoacrylate), poly(ethylcyanoacrylate), poly(butylcyanoacrylate), poly(isobutylcyanoacrylate), poly(isohexylcynaoacrylate), DEAE-methacrylate, DEAE-hexylacrylate, DEAE-acrylamide, DEAE-albumin and DEAE-dextran, polymethylacrylate, polyhexylacrylate, poly(D,L-lactic acid), poly(DL-lactic-co-glycolic acid (PLGA), alginate, and polyethyleneglycol (PEG). Oral formulations for dsRNAs and their preparation are described in detail in U.S. application. Ser. No. 08/886,829 (filed Jul. 1, 1997), Ser. No. 09/108,673 (filed Jul. 1, 1998), Ser. No. 09/256,515 (filed Feb. 23, 1999), Ser. No. 09/082,624 (filed May 21, 1998) and Ser. No. 09/315,298 (filed May 20, 1999), each of which is incorporated herein by reference in their entirety.

Compositions and formulations for parenteral, intrathecal, intraventricular or intrahepatic administration may include sterile aqueous solutions which may also contain buffers, diluents and other suitable additives such as, but not limited to, penetration enhancers, carrier compounds and other pharmaceutically acceptable carriers or excipients.

Pharmaceutical compositions of the present invention include, but are not limited to, solutions, emulsions, and liposome-containing formulations. These compositions may be generated from a variety of components that include, but are not limited to, preformed liquids, self-emulsifying solids and self-emulsifying semisolids. Particularly perfered are formulations that target the liver when treating hepatic disorders such as hepatic carcinoma.

The pharmaceutical formulations of the present invention, which may conveniently be presented in unit dosage form, may be prepared according to conventional techniques well known in the pharmaceutical industry. Such techniques

include the step of bringing into association the active ingredients with the pharmaceutical carrier(s) or excipient(s). In general, the formulations are prepared by uniformly and intimately bringing into association the active ingredients with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

The compositions of the present invention may be formulated into any of many possible dosage forms such as, but not limited to, tablets, capsules, gel capsules, liquid syrups, soft gels, suppositories, and enemas. The compositions of the present invention may also be formulated as suspensions in aqueous, non-aqueous or mixed media. Aqueous suspensions may further contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

Emulsions

The compositions of the present invention may be prepared and formulated as emulsions. Emulsions are typically heterogenous systems of one liquid dispersed in another in the form of droplets usually exceeding 0.1 .mu.m in diameter (Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199; Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., Volume 1, p. 245; Block in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 2, p. 335; Higuchi et al., in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., 1985, p. 301). Emulsions are often biphasic systems comprising two immiscible liquid phases intimately mixed and dispersed with each other. In general, emulsions may be of either the water-in-oil (w/o) or the oil-in-water (o/w) variety. When an aqueous phase is finely divided into and dispersed as minute droplets into a bulk oily phase, the resulting composition is called a water-in-oil (w/o) emulsion. Alternatively,

when an oily phase is finely divided into and dispersed as minute droplets into a bulk aqueous phase, the resulting composition is called an oil-in-water (o/w) emulsion. Emulsions may contain additional components in addition to the dispersed phases, and the active drug which may be present as a solution in either the aqueous phase, oily phase or itself as a separate phase. Pharmaceutical excipients such as emulsifiers, stabilizers, dyes, and anti-oxidants may also be present in emulsions as needed. Pharmaceutical emulsions may also be multiple emulsions that are comprised of more than two phases such as, for example, in the case of oil-in-water-in-oil (o/w/o) and water-in-oil-in-water (w/o/w) emulsions. Such complex formulations often provide certain advantages that simple binary emulsions do not. Multiple emulsions in which individual oil droplets of an o/w emulsion enclose small water droplets constitute a w/o/w emulsion. Likewise a system of oil droplets enclosed in globules of water stabilized in an oily continuous phase provides an o/w/o emulsion.

Emulsions are characterized by little or no thermodynamic stability. Often, the dispersed or discontinuous phase of the emulsion is well dispersed into the external or continuous phase and maintained in this form through the means of emulsifiers or the viscosity of the formulation. Either of the phases of the emulsion may be a semisolid or a solid, as is the case of emulsion-style ointment bases and creams. Other means of stabilizing emulsions entail the use of emulsifiers that may be incorporated into either phase of the emulsion. Emulsifiers may broadly be classified into four categories: synthetic surfactants, naturally occurring emulsifiers, absorption bases, and finely dispersed solids (Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

Synthetic surfactants, also known as surface active agents, have found wide applicability in the formulation of emulsions and have been reviewed in the literature (Rieger, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), Marcel Dekker,

Inc., New York, N.Y., 1988, volume 1, p. 199). Surfactants are typically amphiphilic and comprise a hydrophilic and a hydrophobic portion. The ratio of the hydrophilic to the hydrophobic nature of the surfactant has been termed the hydrophile/lipophile balance (HLB) and is a valuable tool in categorizing and selecting surfactants in the preparation of formulations. Surfactants may be classified into different classes based on the nature of the hydrophilic group: nonionic, anionic, cationic and amphoteric (Rieger, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 285).

Naturally occurring emulsifiers used in emulsion formulations include lanolin, beeswax, phosphatides, lecithin and acacia. Absorption bases possess hydrophilic properties such that they can soak up water to form w/o emulsions yet retain their semisolid consistencies, such as anhydrous lanolin and hydrophilic petrolatum. Finely divided solids have also been used as good emulsifiers especially in combination with surfactants and in viscous preparations. These include polar inorganic solids, such as heavy metal hydroxides, nonswelling clays such as bentonite, attapulgite, hectorite, kaolin, montmorillonite, colloidal aluminum silicate and colloidal magnesium aluminum silicate, pigments and nonpolar solids such as carbon or glyceryl tristearate.

A large variety of non-emulsifying materials are also included in emulsion formulations and contribute to the properties of emulsions. These include fats, oils, waxes, fatty acids, fatty alcohols, fatty esters, humectants, hydrophilic colloids, preservatives and antioxidants (Block, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199).

Hydrophilic colloids or hydrocolloids include naturally occurring gums and synthetic polymers such as polysaccharides (for example, acacia, agar, alginic acid, carrageenan, guar gum, karaya gum, and tragacanth), cellulose derivatives (for

example, carboxymethylcellulose and carboxypropylcellulose), and synthetic polymers (for example, carbomers, cellulose ethers, and carboxyvinyl polymers). These disperse or swell in water to form colloidal solutions that stabilize emulsions by forming strong interfacial films around the dispersed-phase droplets and by increasing the viscosity of the external phase.

Since emulsions often contain a number of ingredients such as carbohydrates, proteins, sterols and phosphatides that may readily support the growth of microbes, these formulations often incorporate preservatives. Commonly used preservatives included in emulsion formulations include methyl paraben, propyl paraben, quaternary ammonium salts, benzalkonium chloride, esters of p-hydroxybenzoic acid, and boric acid. Antioxidants are also commonly added to emulsion formulations to prevent deterioration of the formulation. Antioxidants used may be free radical scavengers such as tocopherols, alkyl gallates, butylated hydroxyanisole, butylated hydroxytoluene, or reducing agents such as ascorbic acid and sodium metabisulfite, and antioxidant synergists such as citric acid, tartaric acid, and lecithin.

The application of emulsion formulations via dermatological, oral and parenteral routes and methods for their manufacture have been reviewed in the literature (Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199). Emulsion formulations for oral delivery have been very widely used because of ease of formulation, as well as efficacy from an absorption and bioavailability standpoint (Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245; Idson, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 199). Mineral-oil base laxatives, oil-soluble vitamins and high fat nutritive preparations are among the materials that have commonly been administered orally as o/w emulsions.

In one embodiment of the present invention, the compositions of dsRNAs and nucleic acids are formulated as microemulsions. A microemulsion may be defined as a system of water, oil and amphiphile which is a single optically isotropic and thermodynamically stable liquid solution (Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245). Typically microemulsions are systems that are prepared by first dispersing an oil in an aqueous surfactant solution and then adding a sufficient amount of a fourth component, generally an intermediate chain-length alcohol to form a transparent system. Therefore, microemulsions have also been described as thermodynamically stable, isotropically clear dispersions of two immiscible liquids that are stabilized by interfacial films of surface-active molecules (Leung and Shah, in: Controlled Release of Drugs: Polymers and Aggregate Systems, Rosoff, M., Ed., 1989, VCH Publishers, New York, pages 185-215). Microemulsions commonly are prepared via a combination of three to five components that include oil, water, surfactant, cosurfactant and electrolyte. Whether the microemulsion is of the water-in-oil (w/o) or an oil-in-water (o/w) type is dependent on the properties of the oil and surfactant used and on the structure and geometric packing of the polar heads and hydrocarbon tails of the surfactant molecules (Schott, in Remington's Pharmaceutical Sciences, Mack Publishing Co., Easton, Pa., 1985, p. 271).

The phenomenological approach utilizing phase diagrams has been extensively studied and has yielded a comprehensive knowledge, to one skilled in the art, of how to formulate microemulsions (Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245; Block, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 335). Compared to conventional emulsions, microemulsions offer the advantage of solubilizing water-insoluble drugs in a formulation of thermodynamically stable droplets that are formed spontaneously.

Surfactants used in the preparation of microemulsions include, but are not limited to, ionic surfactants, non-ionic surfactants, Brij 96, polyoxyethylene oleyl ethers, polyglycerol fatty acid esters, tetraglycerol monolaurate (ML310), tetraglycerol monooleate (MO310), hexaglycerol monooleate (PO310), hexaglycerol pentaoleate (PO500), decaglycerol monocaprate (MCA750), decaglycerol monooleate (MO750), decaglycerol sequioleate (SO750), decaglycerol decaoleate (DAO750), alone or in combination with cosurfactants. The cosurfactant, usually a short-chain alcohol such as ethanol, 1-propanol, and 1-butanol, serves to increase the interfacial fluidity by penetrating into the surfactant film and consequently creating a disordered film because of the void space generated among surfactant molecules. Microemulsions may, however, be prepared without the use of cosurfactants and alcohol-free self-emulsifying microemulsion systems are known in the art. The aqueous phase may typically be, but is not limited to, water, an aqueous solution of the drug, glycerol, PEG300, PEG400, polyglycerols, propylene glycols, and derivatives of ethylene glycol. The oil phase may include, but is not limited to, materials such as Captex 300, Captex 355, Capmul MCM, fatty acid esters, medium chain (C8-C12) mono, di, and tri-glycerides, polyoxyethylated glyceryl fatty acid esters, fatty alcohols, polyglycolized glycerides, saturated polyglycolized C8-C10 glycerides, vegetable oils and silicone oil.

Microemulsions are particularly of interest from the standpoint of drug solubilization and the enhanced absorption of drugs. Lipid based microemulsions (both o/w and w/o) have been proposed to enhance the oral bioavailability of drugs, including peptides (Constantinides et al., Pharmaceutical Research, 1994, 11, 1385-1390; Ritschel, Meth. Find. Exp. Clin. Pharmacol., 1993, 13, 205). Microemulsions afford advantages of improved drug solubilization, protection of drug from enzymatic hydrolysis, possible enhancement of drug absorption due to surfactant-induced alterations in membrane fluidity and permeability, ease of preparation, ease of oral administration over solid dosage forms, improved clinical potency, and decreased toxicity (Constantinides et al., Pharmaceutical Research, 1994, 11, 1385; Ho et al., J.

Pharm. Sci., 1996, 85, 138-143). Often microemulsions may form spontaneously when their components are brought together at ambient temperature. This may be particularly advantageous when formulating thermolabile drugs, peptides or dsRNAs. Microemulsions have also been effective in the transdermal delivery of active components in both cosmetic and pharmaceutical applications. It is expected that the microemulsion compositions and formulations of the present invention will facilitate the increased systemic absorption of dsRNAs and nucleic acids from the gastrointestinal tract, as well as improve the local cellular uptake of dsRNAs and nucleic acids.

Microemulsions of the present invention may also contain additional components and additives such as sorbitan monostearate (Grill 3), Labrasol, and penetration enhancers to improve the properties of the formulation and to enhance the absorption of the dsRNAs and nucleic acids of the present invention. Penetration enhancers used in the microemulsions of the present invention may be classified as belonging to one of five broad categories--surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p. 92). Each of these classes has been discussed above.

Liposomes

There are many organized surfactant structures besides microemulsions that have been studied and used for the formulation of drugs. These include monolayers, micelles, bilayers and vesicles. Vesicles, such as liposomes, have attracted great interest because of their specificity and the duration of action they offer from the standpoint of drug delivery. As used in the present invention, the term "liposome" means a vesicle composed of amphiphilic lipids arranged in a spherical bilayer or bilayers.

Liposomes are unilamellar or multilamellar vesicles which have a membrane formed from a lipophilic material and an aqueous interior. The aqueous portion

contains the composition to be delivered. Cationic liposomes possess the advantage of being able to fuse to the cell wall. Non-cationic liposomes, although not able to fuse as efficiently with the cell wall, are taken up by macrophages in vivo.

In order to cross intact mammalian skin, lipid vesicles must pass through a series of fine pores, each with a diameter less than 50 nm, under the influence of a suitable transdermal gradient. Therefore, it is desirable to use a liposome which is highly deformable and able to pass through such fine pores.

Further advantages of liposomes include; liposomes obtained from natural phospholipids are biocompatible and biodegradable; liposomes can incorporate a wide range of water and lipid soluble drugs; liposomes can protect encapsulated drugs in their internal compartments from metabolism and degradation (Rosoff, in Pharmaceutical Dosage Forms, Lieberman, Rieger and Banker (Eds.), 1988, Marcel Dekker, Inc., New York, N.Y., volume 1, p. 245). Important considerations in the preparation of liposome formulations are the lipid surface charge, vesicle size and the aqueous volume of the liposomes.

Liposomes are useful for the transfer and delivery of active ingredients to the site of action. Because the liposomal membrane is structurally similar to biological membranes, when liposomes are applied to a tissue, the liposomes start to merge with the cellular membranes and as the merging of the liposome and cell progresses, the liposomal contents are emptied into the cell where the active agent may act.

Liposomal formulations have been the focus of extensive investigation as the mode of delivery for many drugs. There is growing evidence that for topical administration, liposomes present several advantages over other formulations. Such advantages include reduced side-effects related to high systemic absorption of the administered drug, increased accumulation of the administered drug at the desired

target, and the ability to administer a wide variety of drugs, both hydrophilic and hydrophobic, into the skin.

Several reports have detailed the ability of liposomes to deliver agents including high-molecular weight DNA into the skin. Compounds including analgesics, antibodies, hormones and high-molecular weight DNAs have been administered to the skin. The majority of applications resulted in the targeting of the upper epidermis

Liposomes fall into two broad classes. Cationic liposomes are positively charged liposomes which interact with the negatively charged DNA molecules to form a stable complex. The positively charged DNA/liposome complex binds to the negatively charged cell surface and is internalized in an endosome. Due to the acidic pH within the endosome, the liposomes are ruptured, releasing their contents into the cell cytoplasm (Wang et al., Biochem. Biophys. Res. Commun., 1987, 147, 980-985).

Liposomes which are pH-sensitive or negatively-charged, entrap DNA rather than complex with it. Since both the DNA and the lipid are similarly charged, repulsion rather than complex formation occurs. Nevertheless, some DNA is entrapped within the aqueous interior of these liposomes. pH-sensitive liposomes have been used to deliver DNA encoding the thymidine kinase gene to cell monolayers in culture. Expression of the exogenous gene was detected in the target cells (Zhou et al., Journal of Controlled Release, 1992, 19, 269-274).

One major type of liposomal composition includes phospholipids other than naturally-derived phosphatidylcholine. Neutral liposome compositions, for example, can be formed from dimyristoyl phosphatidylcholine (DMPC) or dipalmitoyl phosphatidylcholine (DPPC). Anionic liposome compositions generally are formed from dimyristoyl phosphatidylglycerol, while anionic fusogenic liposomes are formed primarily from dioleoyl phosphatidylethanolamine (DOPE). Another type of liposomal composition is formed from phosphatidylcholine (PC) such as, for example,

soybean PC, and egg PC. Another type is formed from mixtures of phospholipid and/or phosphatidylcholine and/or cholesterol.

Several studies have assessed the topical delivery of liposomal drug formulations to the skin. Application of liposomes containing interferon to guinea pig skin resulted in a reduction of skin herpes sores while delivery of interferon via other means (e.g. as a solution or as an emulsion) were ineffective (Weiner et al., Journal of Drug Targeting, 1992, 2, 405-410). Further, an additional study tested the efficacy of interferon administered as part of a liposomal formulation to the administration of interferon using an aqueous system, and concluded that the liposomal formulation was superior to aqueous administration (du Plessis et al., Antiviral Research, 1992, 18, 259-265).

Non-ionic liposomal systems have also been examined to determine their utility in the delivery of drugs to the skin, in particular systems comprising non-ionic surfactant and cholesterol. Non-ionic liposomal formulations comprising Novasome.TM. I (glyceryl dilaurate/cholesterol/po- lyoxyethylene-10-stearyl ether) and Novasome.TM. II (glyceryl distearate/cholesterol/polyoxyethylene-10-stearyl ether) were used to deliver cyclosporin-A into the dermis of mouse skin. Results indicated that such non-ionic liposomal systems were effective in facilitating the deposition of cyclosporin-A into different layers of the skin (Hu et al. S.T.P.Pharma. Sci., 1994, 4, 6, 466).

Liposomes also include "sterically stabilized" liposomes, a term which, as used herein, refers to liposomes comprising one or more specialized lipids that, when incorporated into liposomes, result in enhanced circulation lifetimes relative to liposomes lacking such specialized lipids. Examples of sterically stabilized liposomes are those in which part of the vesicle-forming lipid portion of the liposome (A) comprises one or more glycolipids, such as monosialoganglioside G.sub.M1, or (B) is derivatized with one or more hydrophilic polymers, such as a polyethylene glycol

(PEG) moiety. While not wishing to be bound by any particular theory, it is thought in the art that, at least for sterically stabilized liposomes containing gangliosides, sphingomyelin, or PEG-derivatized lipids, the enhanced circulation half-life of these sterically stabilized liposomes derives from a reduced uptake into cells of the reticuloendothelial system (RES) (Allen et al., FEBS Letters, 1987, 223, 42; Wu et al., Cancer Research, 1993, 53, 3765).

Various liposomes comprising one or more glycolipids are known in the art. Papahadjopoulos et al. (Ann. N.Y. Acad. Sci., 1987, 507, 64) reported the ability of monosialoganglioside G.sub.M1, galactocerebroside sulfate and phosphatidylinositol to improve blood half-lives of liposomes. These findings were expounded upon by Gabizon et al. (Proc. Natl. Acad. Sci. U.S.A., 1988, 85, 6949). U.S. Pat. No. 4,837,028 and WO 88/04924, both to Allen et al., disclose liposomes comprising (1) sphingomyelin and (2) the ganglioside G.sub.M1 or a galactocerebroside sulfate ester. U.S. Pat. No. 5,543,152 (Webb et al.) discloses liposomes comprising sphingomyelin. Liposomes comprising 1,2-sn-dimyristoylphosphat- idylcholine are disclosed in WO 97/13499 (Lim et al).

Many liposomes comprising lipids derivatized with one or more hydrophilic polymers, and methods of preparation thereof, are known in the art. Sunamoto et al. (Bull. Chem. Soc. Jpn., 1980, 53, 2778) described liposomes comprising a nonionic detergent, 2C.sub.1215G, that contains a PEG moiety. Illum et al. (FEBS Lett., 1984, 167, 79) noted that hydrophilic coating of polystyrene particles with polymeric glycols results in significantly enhanced blood half-lives. Synthetic phospholipids modified by the attachment of carboxylic groups of polyalkylene glycols (e.g., PEG) are described by Sears (U.S. Pat. Nos. 4,426,330 and 4,534,899). Klibanov et al. (FEBS Lett., 1990, 268, 235) described experiments demonstrating that liposomes comprising phosphatidylethanolamine (PE) derivatized with PEG or PEG stearate have significant increases in blood circulation half-lives. Blume et al. (Biochimica et Biophysica Acta, 1990, 1029, 91) extended such observations to other PEG-derivatized phospholipids,

e.g., DSPE-PEG, formed from the combination of distearoylphosphatidylethanolamine (DSPE) and PEG. Liposomes having covalently bound PEG moieties on their external surface are described in European Patent No. EP 0 445 131 B1 and WO 90/04384 to Fisher. Liposome compositions containing 1-20 mole percent of PE derivatized with PEG, and methods of use thereof, are described by Woodle et al. (U.S. Pat. Nos. 5,013,556 and 5,356,633) and Martin et al. (U.S. Pat. No. 5,213,804 and European Patent No. EP 0 496 813 B1). Liposomes comprising a number of other lipid-polymer conjugates are disclosed in WO 91/05545 and U.S. Pat. No. 5,225,212 (both to Martin et al.) and in WO 94/20073 (Zalipsky et al.) Liposomes comprising PEG-modified ceramide lipids are described in WO 96/10391 (Choi et al). U.S. Pat. No. 5,540,935 (Miyazaki et al.) and U.S. Pat. No. 5,556,948 (Tagawa et al.) describe PEG-containing liposomes that can be further derivatized with functional moieties on their surfaces.

A limited number of liposomes comprising nucleic acids are known in the art. WO 96/40062 to Thierry et al. discloses methods for encapsulating high molecular weight nucleic acids in liposomes. U.S. Pat. No. 5,264,221 to Tagawa et al. discloses protein-bonded liposomes and asserts that the contents of such liposomes may include an dsRNA RNA. U.S. Pat. No. 5,665,710 to Rahman et al. describes certain methods of encapsulating oligodeoxynucleotides in liposomes. WO 97/04787 to Love et al. discloses liposomes comprising dsRNA dsRNAs targeted to the raf gene.

Transfersomes are yet another type of liposomes, and are highly deformable lipid aggregates which are attractive candidates for drug delivery vehicles. Transfersomes may be described as lipid droplets which are so highly deformable that they are easily able to penetrate through pores which are smaller than the droplet. Transfersomes are adaptable to the environment in which they are used, e.g. they are self-optimizing (adaptive to the shape of pores in the skin), self-repairing, frequently reach their targets without fragmenting, and often self-loading. To make transfersomes it is possible to add surface edge-activators, usually surfactants, to a standard

liposomal composition. Transfersomes have been used to deliver serum albumin to the skin. The transfersome-mediated delivery of serum albumin has been shown to be as effective as subcutaneous injection of a solution containing serum albumin.

Surfactants find wide application in formulations such as emulsions (including microemulsions) and liposomes. The most common way of classifying and ranking the properties of the many different types of surfactants, both natural and synthetic, is by the use of the hydrophile/lipophile balance (HLB). The nature of the hydrophilic group (also known as the "head") provides the most useful means for categorizing the different surfactants used in formulations (Rieger, in Pharmaceutical Dosage Forms, Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

If the surfactant molecule is not ionized, it is classified as a nonionic surfactant. Nonionic surfactants find wide application in pharmaceutical and cosmetic products and are usable over a wide range of pH values. In general their HLB values range from 2 to about 18 depending on their structure. Nonionic surfactants include nonionic esters such as ethylene glycol esters, propylene glycol esters, glyceryl esters, polyglyceryl esters, sorbitan esters, sucrose esters, and ethoxylated esters. Nonionic alkanolamides and ethers such as fatty alcohol ethoxylates, propoxylated alcohols, and ethoxylated/propoxylated block polymers are also included in this class. The polyoxyethylene surfactants are the most popular members of the nonionic surfactant class.

If the surfactant molecule carries a negative charge when it is dissolved or dispersed in water, the surfactant is classified as anionic. Anionic surfactants include carboxylates such as soaps, acyl lactylates, acyl amides of amino acids, esters of sulfuric acid such as alkyl sulfates and ethoxylated alkyl sulfates, sulfonates such as alkyl benzene sulfonates, acyl isethionates, acyl taurates and sulfosuccinates, and phosphates. The most important members of the anionic surfactant class are the alkyl sulfates and the soaps.

If the surfactant molecule carries a positive charge when it is dissolved or dispersed in water, the surfactant is classified as cationic. Cationic surfactants include quaternary ammonium salts and ethoxylated amines. The quaternary ammonium salts are the most used members of this class.

If the surfactant molecule has the ability to carry either a positive or negative charge, the surfactant is classified as amphoteric. Amphoteric surfactants include acrylic acid derivatives, substituted alkylamides, N-alkylbetaines and phosphatides.

The use of surfactants in drug products, formulations and in emulsions has been reviewed (Rieger, in Pharmaceutical Dosage Forms, Marcel Dekker, Inc., New York, N.Y., 1988, p. 285).

Penetration Enhancers

In one embodiment, the present invention employs various penetration enhancers to effect the efficient delivery of nucleic acids, particularly dsRNAs, to the skin of animals. Most drugs are present in solution in both ionized and nonionized forms. However, usually only lipid soluble or lipophilic drugs readily cross cell membranes. It has been discovered that even non-lipophilic drugs may cross cell membranes if the membrane to be crossed is treated with a penetration enhancer. In addition to aiding the diffusion of non-lipophilic drugs across cell membranes, penetration enhancers also enhance the permeability of lipophilic drugs.

Penetration enhancers may be classified as belonging to one of five broad categories, i.e., surfactants, fatty acids, bile salts, chelating agents, and non-chelating non-surfactants (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p.92). Each of the above mentioned classes of penetration enhancers are described below in greater detail.

Surfactants: In connection with the present invention, surfactants (or "surface-active agents") are chemical entities which, when dissolved in an aqueous solution,

reduce the surface tension of the solution or the interfacial tension between the aqueous solution and another liquid, with the result that absorption of dsRNAs through the mucosa is enhanced. In addition to bile salts and fatty acids, these penetration enhancers include, for example, sodium lauryl sulfate, polyoxyethylene-9-lauryl ether and polyoxyethylene-20-cetyl ether) (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, p.92); and perfluorochemical emulsions, such as FC-43. Takahashi et al., J. Pharm. Pharmacol., 1988, 40, 252).

Fatty acids: Various fatty acids and their derivatives which act as penetration enhancers include, for example, oleic acid, lauric acid, capric acid (n-decanoic acid), myristic acid, palmitic acid, stearic acid, linoleic acid, linolenic acid, dicaprate, tricaprate, monoolein (1-monooleoyl-rac-glycerol), dilaurin, caprylic acid, arachidonic acid, glycerol 1-monocaprate, 1-dodecylazacycloheptan-2-one, acylcarnitines, acylcholines, C.sub.1-10 alkyl esters thereof (e.g., methyl, isopropyl and t-butyl), and mono- and di-glycerides thereof (i.e., oleate, laurate, caprate, myristate, palmitate, stearate, linoleate, etc.) (Lee et al., Critical Reviews in Therapeutic Drug Carryier Systems, 1991, p.92; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; El Hariri et al., J. Pharm. Pharmacol., 1992, 44, 651-654).

Bile salts: The physiological role of bile includes the facilitation of dispersion and absorption of lipids and fat-soluble vitamins (Brunton, Chapter 38 in: Goodman & Gilman's The Pharmacological Basis of Therapeutics, 9th Ed., Hardman et al. Eds., McGraw-Hill, New York, 1996, pp. 934-935). Various natural bile salts, and their synthetic derivatives, act as penetration enhancers. Thus the term "bile salts" includes any of the naturally occurring components of bile as well as any of their synthetic derivatives. The bile salts of the invention include, for example, cholic acid (or its pharmaceutically acceptable sodium salt, sodium cholate), dehydrocholic acid (sodium dehydrocholate), deoxycholic acid (sodium deoxycholate), glucholic acid (sodium glucholate), glycholic acid (sodium glycocholate), glycodeoxycholic acid (sodium glycodeoxycholate), taurocholic acid (sodium taurocholate),

taurodeoxycholic acid (sodium taurodeoxycholate), chenodeoxycholic acid (sodium chenodeoxycholate), ursodeoxycholic acid (UDCA), sodium tauro-24,25-dihydro-fusidate (STDHF), sodium glycodihydrofusidate and polyoxyethylene-9-lauryl ether (POE) (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92; Swinyard, Chapter 39 In: Remington's Pharmaceutical Sciences, 18th Ed., Gennaro, ed., Mack Publishing Co., Easton, Pa., 1990, pages 782-783; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; Yamamoto et al., J. Pharm. Exp. Ther., 1992, 263, 25; Yamashita et al., J. Pharm. Sci., 1990, 79, 579-583).

Chelating Agents: Chelating agents, as used in connection with the present invention, can be defined as compounds that remove metallic ions from solution by forming complexes therewith, with the result that absorption of dsRNAs through the mucosa is enhanced. With regards to their use as penetration enhancers in the present invention, chelating agents have the added advantage of also serving as DNase inhibitors, as most characterized DNA nucleases require a divalent metal ion for catalysis and are thus inhibited by chelating agents (Jarrett, J. Chromatogr., 1993, 618, 315-339). Chelating agents of the invention include but are not limited to disodium ethylenediaminetetraacetate (EDTA), citric acid, salicylates (e.g., sodium salicylate, 5-methoxysalicylate and homovanilate), N-acyl derivatives of collagen, laureth-9 and N-amino acyl derivatives of beta-diketones (enamines)(Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92; Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33; Buur et al., J. Control Rel., 1990, 14, 43-51).

Non-chelating non-surfactants: As used herein, non-chelating non-surfactant penetration enhancing compounds can be defined as compounds that demonstrate insignificant activity as chelating agents or as surfactants but that nonetheless enhance absorption of dsRNAs through the alimentary mucosa (Muranishi, Critical Reviews in Therapeutic Drug Carrier Systems, 1990, 7, 1-33). This class of penetration enhancers

include, for example, unsaturated cyclic ureas, 1-alkyl- and 1-alkenylazacyclo-alkanone derivatives (Lee et al., Critical Reviews in Therapeutic Drug Carrier Systems, 1991, page 92); and non-steroidal anti-inflammatory agents such as diclofenac sodium, indomethacin and phenylbutazone (Yamashita et al., J. Pharm. Pharmacol., 1987, 39, 621-626).

Agents that enhance uptake of dsRNAs at the cellular level may also be added to the pharmaceutical and other compositions of the present invention. For example, cationic lipids, such as lipofectin (Junichi et al, U.S. Pat. No. 5,705,188), cationic glycerol derivatives, and polycationic molecules, such as polylysine (Lollo et al., PCT Application WO 97/30731), are also known to enhance the cellular uptake of dsRNAs.

Other agents may be utilized to enhance the penetration of the administered nucleic acids, including glycols such as ethylene glycol and propylene glycol, pyrrols such as 2-pyrrol, azones, and terpenes such as limonene and menthone.

Carriers

Certain compositions of the present invention also incorporate carrier compounds in the formulation. As used herein, "carrier compound" or "carrier" can refer to a nucleic acid, or analog thereof, which is inert (i.e., does not possess biological activity per se) but is recognized as a nucleic acid by in vivo processes that reduce the bioavailability of a nucleic acid having biological activity by, for example, degrading the biologically active nucleic acid or promoting its removal from circulation. The coadministration of a nucleic acid and a carrier compound, typically with an excess of the latter substance, can result in a substantial reduction of the amount of nucleic acid recovered in the liver, kidney or other extracirculatory reservoirs, presumably due to competition between the carrier compound and the nucleic acid for a common receptor. For example, the recovery of a partially phosphorothioate dsRNA in hepatic tissue can be reduced when it is coadministered

with polyinosinic acid, dextran sulfate, polycytidic acid or 4-acetamido-4'isothiocyano-stilbene-2,2'-disulfonic acid (Miyao et al., DsRNA Res. Dev., 1995, 5, 115-121; Takakura et al., DsRNA & Nucl. Acid Drug Dev., 1996, 6, 177-183.

Excipients

In contrast to a carrier compound, a "pharmaceutical carrier" or "excipient" is a pharmaceutically acceptable solvent, suspending agent or any other pharmacologically inert vehicle for delivering one or more nucleic acids to an animal. The excipient may be liquid or solid and is selected, with the planned manner of administration in mind, so as to provide for the desired bulk, consistency, etc., when combined with a nucleic acid and the other components of a given pharmaceutical composition. Typical pharmaceutical carriers include, but are not limited to, binding agents (e.g., pregelatinized maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose, etc.); fillers (e.g., lactose and other sugars, microcrystalline cellulose, pectin, gelatin, calcium sulfate, ethyl cellulose, polyacrylates or calcium hydrogen phosphate, etc.); lubricants (e.g., magnesium stearate, talc, silica, colloidal silicon dioxide, stearic acid, metallic stearates, hydrogenated vegetable oils, corn starch, polyethylene glycols, sodium benzoate, sodium acetate, etc.); disintegrants (e.g., starch, sodium starch glycolate, etc.); and wetting agents (e.g., sodium lauryl sulphate, etc).

Pharmaceutically acceptable organic or inorganic excipient suitable for non-parenteral administration which do not deleteriously react with nucleic acids can also be used to formulate the compositions of the present invention. Suitable pharmaceutically acceptable carriers include, but are not limited to, water, salt solutions, alcohols, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone and the like.

Formulations for topical administration of nucleic acids may include sterile and non-sterile aqueous solutions, non-aqueous solutions in common solvents such as

alcohols, or solutions of the nucleic acids in liquid or solid oil bases. The solutions may also contain buffers, diluents and other suitable additives. Pharmaceutically acceptable organic or inorganic excipients suitable for non-parenteral administration which do not deleteriously react with nucleic acids can be used.

Suitable pharmaceutically acceptable excipients include, but are not limited to, water, salt solutions, alcohol, polyethylene glycols, gelatin, lactose, amylose, magnesium stearate, talc, silicic acid, viscous paraffin, hydroxymethylcellulose, polyvinylpyrrolidone and the like.

Other Components

The compositions of the present invention may additionally contain other adjunct components conventionally found in pharmaceutical compositions, at their art-established usage levels. Thus, for example, the compositions may contain additional, compatible, pharmaceutically-active materials such as, for example, antipruritics, astringents, local anesthetics or anti-inflammatory agents, or may contain additional materials useful in physically formulating various dosage forms of the compositions of the present invention, such as dyes, flavoring agents, preservatives, antioxidants, opacifiers, thickening agents and stabilizers. However, such materials, when added, should not unduly interfere with the biological activities of the components of the compositions of the present invention. The formulations can be sterilized and, if desired, mixed with auxiliary agents, e.g., lubricants, preservatives, stabilizers, wetting agents, emulsifiers, salts for influencing osmotic pressure, buffers, colorings, flavorings and/or aromatic substances and the like which do not deleteriously interact with the nucleic acid(s) of the formulation.

Aqueous suspensions may contain substances which increase the viscosity of the suspension including, for example, sodium carboxymethylcellulose, sorbitol and/or dextran. The suspension may also contain stabilizers.

Certain embodiments of the invention provide pharmaceutical compositions containing (a) one or more antisense compounds and (b) one or more other chemotherapeutic agents which function by a non-antisense mechanism. Examples of such chemotherapeutic agents include but are not limited to daunorubicin, daunomycin, dactinomycin, doxorubicin, epirubicin, idarubicin, esorubicin, bleomycin, mafosfamide, ifosfamide, cytosine arabinoside, bis-chloroethylnitrosurea, busulfan, mitomycin C, actinomycin D, mithramycin, prednisone, hydroxyprogesterone, testosterone, tamoxifen, dacarbazine, procarbazine, hexamethylmelamine, pentamethylmelamine, mitoxantrone, amsacrine, chlorambucil, methylcyclohexylnitrosurea, nitrogen mustards, melphalan, cyclophosphamide, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-azacytidine, hydroxyurea, deoxycoformycin, 4-hydroxyperoxycyclophosphor- amide, 5-fluorouracil (5-FU), 5-fluorodeoxyuridine (5-FUdR), methotrexate (MTX), colchicine, taxol, vincristine, vinblastine, etoposide (VP-16), trimetrexate, irinotecan, topotecan, gemcitabine, teniposide, cisplatin and diethylstilbestrol (DES). See, generally, The Merck Manual of Diagnosis and Therapy, 15th Ed. 1987, pp. 1206-1228, Berkow et al., eds., Rahway, N.J. When used with the compounds of the invention, such chemotherapeutic agents may be used individually (e.g., 5-FU and oligonucleotide), sequentially (e.g., 5-FU and oligonucleotide for a period of time followed by MTX and oligonucleotide), or in combination with one or more other such chemotherapeutic agents (e.g., 5-FU, MTX and oligonucleotide, or 5-FU, radiotherapy and oligonucleotide). Anti-inflammatory drugs, including but not limited to nonsteroidal anti-inflammatory drugs and corticosteroids, and antiviral drugs, including but not limited to ribivirin, vidarabine, acyclovir and ganciclovir, may also be combined in compositions of the invention. See, generally, The Merck Manual of Diagnosis and Therapy, 15th Ed., Berkow et al., eds., 1987, Rahway, N.J., pages 2499-2506 and 46-49, respectively). Other non-antisense chemotherapeutic agents are also within the scope of this invention. Two or more combined compounds may be used together or sequentially.

Toxicity and therapeutic efficacy of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., for determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD50/ED50. Compounds which exhibit high therapeutic indices are preferred.

The data obtained from cell culture assays and animal studies can be used in formulation a range of dosage for use in humans. The dosage of compositions of the invention lies generally within a range of circulating concentrations that include the ED50 with little or no toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized. For any compound used in the method of the invention, the therapeutically effective dose can be estimated initially from cell culture assays. A dose may be formulated in animal models to achieve a circulating plasma concentration range of the compound or, when appropriate, of the polypeptide product of a target sequence (e.g., achieving a decreased concentration of the polypeptide) that includes the IC50 (i.e., the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Such information can be used to more accurately determine useful doses in humans. Levels in plasma may be measured, for example, by high performance liquid chromatography.

In addition to their administration individually or as a plurality, as discussed above, the dsRNAs of the invention can be administered in combination with other known agents effective in treatment of pathological processes mediated by Eg5 expression. In any event, the administering physician can adjust the amount and timing of dsRNA administration on the basis of results observed using standard measures of efficacy known in the art or described herein.

Methods for treating diseases caused by expression of the Eg5 gene

The invention relates in particular to the use of a dsRNA or a pharmaceutical composition prepared therefrom for the treatment of cancer, e.g., for inhibiting tumor growth and tumor metastasis. For example, the dsRNA or a pharmaceutical composition prepared therefrom may be used for the treatment of solid tumors, like breast cancer, lung cancer, head and neck cancer, brain cancer, abdominal cancer, colon cancer, colorectal cancer, esophagus cancer, gastrointestinal cancer, glioma, liver cancer, tongue cancer, neuroblastoma, osteosarcoma, ovarian cancer, pancreatic cancer, prostate cancer, retinoblastoma, Wilm's tumor, multiple myeloma and for the treatment of skin cancer, like melanoma, for the treatment of lymphomas and blood cancer. The invention further relates to the use of an dsRNA according to the invention or a pharmaceutical composition prepared therefrom for inhibiting eg5 expression and/or for inhibiting accumulation of ascites fluid and pleural effusion in different types of cancer, e.g., breast cancer, lung cancer, head cancer, neck cancer, brain cancer, abdominal cancer, colon cancer, colorectal cancer, esophagus cancer, gastrointestinal cancer, glioma, liver cancer, tongue cancer, neuroblastoma, osteosarcoma, ovarian cancer, pancreatic cancer, prostate cancer, retinoblastoma, Wilm's tumor, multiple myeloma, skin cancer, melanoma, lymphomas and blood cancer. Owing to the inhibitory effect on eg5 expression, an dsRNA according to the invention or a pharmaceutical composition prepared therefrom can enhance the quality of life.

The invention furthermore relates to the use of an dsRNA or a pharmaceutical composition thereof, e.g., for treating cancer or for preventing tumor metastasis, in combination with other pharmaceuticals and/or other therapeutic methods, e.g., with known pharmaceuticals and/or known therapeutic methods, such as, for example, those which are currently employed for treating cancer and/or for preventing tumor metastasis. Preference is given to a combination with radiation therapy and chemotherapeutic agents, such as cisplatin, cyclophosphamide, 5-fluorouracil,

adriamycin, daunorubicin or tamoxifen. Other emobiments include the use of a second dsRNA used to inhibit the expression of VEGF.

The invention can also be practiced by including with a specific RNAi agent, in combination with another anti-cancer chemotherapeutic agent, such as any conventional chemotherapeutic agent, or another dsRNA used to inhibt the expression of VEGF. The combination of a specific binding agent with such other agents can potentiate the chemotherapeutic protocol. Numerous chemotherapeutic protocols will present themselves in the mind of the skilled practitioner as being capable of incorporation into the method of the invention. Any chemotherapeutic agent can be used, including alkylating agents, antimetabolites, hormones and antagonists, radioisotopes, as well as natural products. For example, the compound of the invention can be administered with antibiotics such as doxorubicin and other anthracycline analogs, nitrogen mustards such as cyclophosphamide, pyrimidine analogs such as 5-fluorouracil, cisplatin, hydroxyurea, taxol and its natural and synthetic derivatives, and the like. As another example, in the case of mixed tumors, such as adenocarcinoma of the breast, where the tumors include gonadotropin-dependent and gonadotropin-independent cells, the compound can be administered in conjunction with leuprolide or goserelin (synthetic peptide analogs of LH-RH). Other antineoplastic protocols include the use of a tetracycline compound with another treatment modality, e.g., surgery, radiation, etc., also referred to herein as "adjunct antineoplastic modalities." Thus, the method of the invention can be employed with such conventional regimens with the benefit of reducing side effects and enhancing efficacy.

Methods for inhibiting expression of the Eg5 gene

In yet another aspect, the invention provides a method for inhibiting the expression of the Eg5 gene in a mammal. The method comprises administering a composition of the invention to the mammal such that expression of the target Eg5

gene is silenced. Because of their high specificity, the dsRNAs of the invention specifically target RNAs (primary or processed) of the target Eg5 gene. Compositions and methods for inhibiting the expression of these Eg5 genes using dsRNAs can be performed as described elsewhere herein.

In one embodiment, the method comprises administering a composition comprising a dsRNA, wherein the dsRNA comprises a nucleotide sequence which is complementary to at least a part of an RNA transcript of the Eg5 gene of the mammal to be treated. When the organism to be treated is a mammal such as a human, the composition may be administered by any means known in the art including, but not limited to oral or parenteral routes, including intravenous, intramuscular, subcutaneous, transdermal, airway (aerosol), nasal, rectal, and topical (including buccal and sublingual) administration. In preferred embodiments, the compositions are administered by intravenous infusion or injection.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the invention, suitable methods and materials are described below. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and not intended to be limiting.

EXAMPLES

Gene Walking of the Eg5 gene

Initial Screening set

siRNA design was carried out to identify siRNAs targeting Eg5 (also known as KIF11, HSKP, KNSL1 and TRIP5). Human mRNA sequences to Eg5, RefSeq ID number:NM_004523, was used.

siRNA duplexes cross-reactive to human and mouse Eg5 were designed. Twenty-four duplexes were synthesized for screening. (Table 1).

Expanded screening set

A second screening set was defined with 266 siRNAs targeting human EG5, as well as its rhesus monkey ortholog (Table 2). An expanded screening set was selected with 328 siRNA targeting human EG5, with no necessity to hit any EG5 mRNA of other species (Table 3).

The sequences for human and a partial rhesus EG5 mRNAs were downloaded from NCBI Nucleotide database and the human sequence was further on used as reference sequence (Human EG5:NM_004523.2, 4908 bp, and Rhesus EG5: XM_001087644.1, 878 bp (only 5' part of human EG5)

For identification of further rhesus EG5 sequences a mega blast search with the human sequence was conducted at NCBI against rhesus reference genome. The downloaded rhesus sequence and the hit regions in the blast hit were assembled to a rhesus consensus sequence with ~92% identity to human EG5 over the full-length.

All possible 19mers were extracted from the human mRNA sequence, resulting in the pool of candidate target sites corresponding to 4890 (sense strand) sequences of human-reactive EG5 siRNAs.

Human-rhesus cross-reactivity as prerequisite for in *silico* selection of siRNAs for an initial screening set out of this candidate pool. To determine rhesus-reactive siRNAs, each candidate siRNA target site was searched for presence in the assembled rhesus sequence. Further, the predicted specificity of the siRNA as criterion for selection of out the pool of human-rhesus cross-reactive siRNAs, manifested by targeting human EG5 mRNA sequences, but not other human mRNAs.

The specificity of an siRNA can be expressed via its potential to target other genes, which are referred to as "off-target genes".

For predicting the off-target potential of an siRNA, the following assumptions were made:

1) off-target potential of a strand can be deduced from the number and distribution of mismatches to an off-target

2) the most relevant off-target, that is the gene predicted to have the highest probability to be silenced due to tolerance of mismatches, determines the off-target potential of the strand

3) positions 2 to 9 (counting 5' to 3') of a strand (seed region) may contribute more to off-target potential than rest of sequence (that is non-seed and cleavage site region)

4) positions 10 and 11 (counting 5' to 3') of a strand (cleavage site region) may contribute more to off-target potential than non-seed region (that is positions 12 to 18, counting 5' to 3')

5) positions 1 and 19 of each strand are not relevant for off-target interactions

6) off-target potential can be expressed by the off-target score of the most relevant off-target, calculated based on number and position of mismatches of the strand to the most homologous region in the off-target gene considering assumptions 3 to 5

7) off-target potential of antisense and sense strand will be relevant, whereas potential abortion of sense strand activity by internal modifications introduced is likely

SiRNAs with low off-target potential were defined as preferable and assumed to be more specific.

In order to identify human EG5-specific siRNAs, all other human transcripts, which were all considered potential off-targets, were searched for potential target regions for human-rhesus cross-reactive 19mer sense strand sequences as well as complementary antisense strands. For this, the fastA algorithm was used to determine the most homologues hit region in each sequence of the human RefSeq database, which we assume to represent the comprehensive human transcriptome.

To rank all potential off-targets according to assumptions 3 to 5, and by this identify the most relevant off-target gene and its off-target score, fastA output files were analyzed further by a perl script.

The script extracted the following off-target properties for each 19mer input sequence and each off-target gene to calculate the off-target score:

Number of mismatches in non-seed region

Number of mismatches in seed region

Number of mismatches in cleavage site region

The off-target score was calculated by considering assumptions 3 to 5 as follows:

Off-target score = number of seed mismatches * 10

+ number of cleavage site mismatches * 1.2

+ number of non-seed mismatches * 1

The most relevant off-target gene for each 19mer sequence was defined as the gene with the lowest off-target score. Accordingly, the lowest off-target score was defined as representative for the off-target potential of a strand.

For the screening set in Table 2, an off-target score of 3 or more for the antisense strand and 2 or more for the sense strand was chosen as prerequisite for selection of siRNAs, whereas all sequences containing 4 or more consecutive G's (poly-G sequences) were excluded. 266 human-rhesus cross-reactive sequences passing the specificity criterion, were selected based on this cut-off (see Table 2).

For definition of the expanded screening set the cross-reactivity to rhesus was disgarded, re-calculated the predicted specificity based on the newly available human RefSeq database and selected only those 328 non-poly-G siRNAs with off-target score of 2,2 or more for the antisense and sense strand (see Table 3).

For the Tables: Key: A,G,C,U-ribonucleotides: T-deoxythymidine: u,c-2'-O-methyl nucleotides: s-phosphorothioate linkage

dsRNA synthesis

Source of reagents

Where the source of a reagent is not specifically given herein, such reagent may be obtained from any supplier of reagents for molecular biology at a quality/purity standard for application in molecular biology.

siRNA synthesis

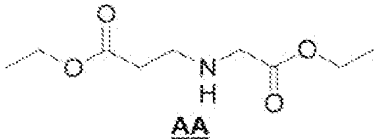
Single-stranded RNAs were produced by solid phase synthesis on a scale of 1 µmole using an Expedite 8909 synthesizer (Applied Biosystems, Applera Deutschland GmbH, Darmstadt, Germany) and controlled pore glass (CPG, 500Å, Proligo Biochemie GmbH, Hamburg, Germany) as solid support. RNA and RNA containing *2'-O*-methyl nucleotides were generated by solid phase synthesis employing the corresponding phosphoramidites and *2'-O*-methyl phosphoramidites, respectively (Proligo Biochemie GmbH, Hamburg, Germany). These building blocks were incorporated at selected sites within the sequence of the oligoribonucleotide chain using standard nucleoside phosphoramidite chemistry such as described in Current protocols in nucleic acid chemistry, Beaucage, S.L. et al. (Edrs.), John Wiley & Sons, Inc., New York, NY, USA. Phosphorothioate linkages were introduced by replacement of the iodine oxidizer solution with a solution of the Beaucage reagent (Chruachem Ltd, Glasgow, UK) in acetonitrile (1%). Further ancillary reagents were obtained from Mallinckrodt Baker (Griesheim, Germany).

Deprotection and purification of the crude oligoribonucleotides by anion exchange HPLC were carried out according to established procedures. Yields and concentrations were determined by UV absorption of a solution of the respective RNA at a wavelength of 260 nm using a spectral photometer (DU 640B, Beckman Coulter GmbH, Unterschleißheim, Germany). Double stranded RNA was generated by mixing an equimolar solution of complementary strands in annealing buffer (20 mM sodium

phosphate, pH 6.8; 100 mM sodium chloride), heated in a water bath at 85 - 90°C for 3 minutes and cooled to room temperature over a period of 3 - 4 hours. The annealed RNA solution was stored at –20 °C until use.

For the synthesis of 3'-cholesterol-conjugated siRNAs (herein referred to as -Chol-3'), an appropriately modified solid support was used for RNA synthesis. The modified solid support was prepared as follows:

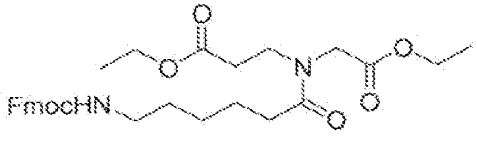
Diethyl-2-azabutane-1,4-dicarboxylate **AA**



AA

A 4.7 M aqueous solution of sodium hydroxide (50 mL) was added into a stirred, ice-cooled solution of ethyl glycinate hydrochloride (32.19 g, 0.23 mole) in water (50 mL). Then, ethyl acrylate (23.1 g, 0.23 mole) was added and the mixture was stirred at room temperature until completion of the reaction was ascertained by TLC. After 19 h the solution was partitioned with dichloromethane (3 x 100 mL). The organic layer was dried with anhydrous sodium sulfate, filtered and evaporated. The residue was distilled to afford AA (28.8 g, 61%).

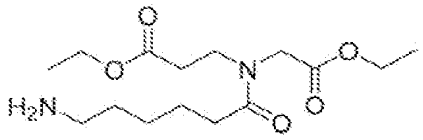
3-{Ethoxycarbonylmethyl-[6-(9H-fluoren-9-ylmethoxycarbonyl-amino)-hexanoyl]-amino}-propionic acid ethyl ester **AB**



AB

Fmoc-6-amino-hexanoic acid (9.12 g, 25.83 mmol) was dissolved in dichloromethane (50 mL) and cooled with ice. Diisopropylcarbodiimde (3.25 g, 3.99 mL, 25.83 mmol) was added to the solution at 0°C. It was then followed by the addition of Diethyl-azabutane-1,4-dicarboxylate (5 g, 24.6 mmol) and dimethylamino pyridine (0.305 g, 2.5 mmol). The solution was brought to room temperature and stirred further for 6 h. Completion of the reaction was ascertained by TLC. The reaction mixture was concentrated under vacuum and ethyl acetate was added to precipitate diisopropyl urea. The suspension was filtered. The filtrate was washed with 5% aqueous hydrochloric acid, 5% sodium bicarbonate and water. The combined organic layer was dried over sodium sulfate and concentrated to give the crude product which was purified by column chromatography (50 % EtOAC/Hexanes) to yield 11.87 g (88%) of AB.

3-[(6-Amino-hexanoyl)-ethoxycarbonylmethyl-amino]-propionic acid ethyl ester **AC**

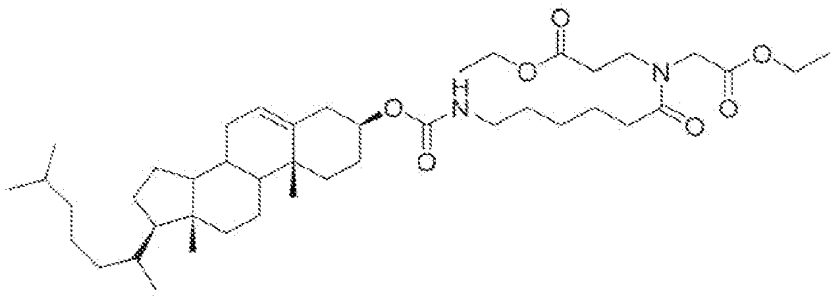


AC

3-{Ethoxycarbonylmethyl-[6-(9H-fluoren-9-ylmethoxycarbonylamino)-hexanoyl]-amino}-propionic acid ethyl ester AB (11.5 g, 21.3 mmol) was dissolved in 20% piperidine in dimethylformamide at 0°C. The solution was continued stirring for 1 h. The reaction mixture was concentrated under vacuum, water was added to the residue, and the product was extracted with ethyl acetate. The crude product was purified by conversion into its hydrochloride salt.

3-({6-[17-(1,5-Dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-

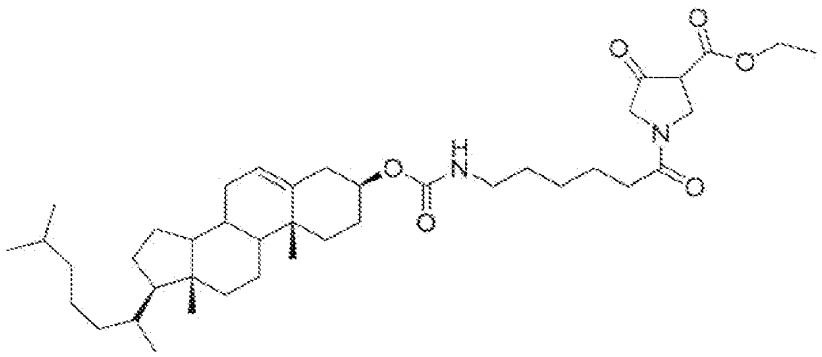
yloxycarbonylamino]-hexanoyl}ethoxycarbonylmethyl-amino)-propionic acid ethyl ester **AD**



AD

The hydrochloride salt of 3-[(6-Amino-hexanoyl)-ethoxycarbonylmethyl-amino]-propionic acid ethyl ester AC (4.7 g, 14.8 mmol) was taken up in dichloromethane. The suspension was cooled to 0°C on ice. To the suspension diisopropylethylamine (3.87 g, 5.2 mL, 30 mmol) was added. To the resulting solution cholesteryl chloroformate (6.675 g, 14.8 mmol) was added. The reaction mixture was stirred overnight. The reaction mixture was diluted with dichloromethane and washed with 10% hydrochloric acid. The product was purified by flash chromatography (10.3 g, 92%).

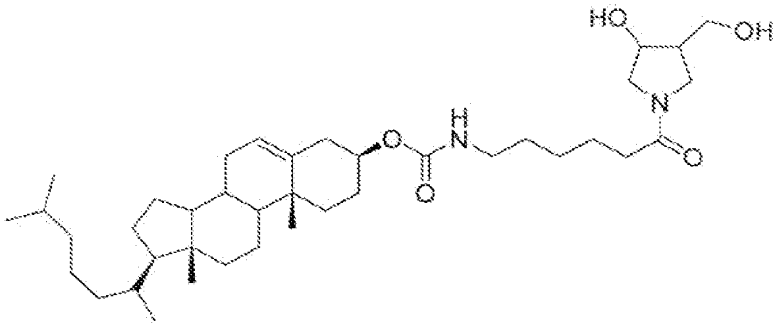
1-{6-[17-(1,5-Dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a] phenanthren-3-yloxycarbonylamino]-hexanoyl}-4-oxo-pyrrolidine-3-carboxylic acid ethyl ester **AE**



AE

Potassium t-butoxide (1.1 g, 9.8 mmol) was slurried in 30 mL of dry toluene. The mixture was cooled to 0°C on ice and 5 g (6.6 mmol) of diester AD was added slowly with stirring within 20 mins. The temperature was kept below 5°C during the addition. The stirring was continued for 30 mins at 0°C and 1 mL of glacial acetic acid was added, immediately followed by 4 g of $NaH_2PO_4 \cdot H_2O$ in 40 mL of water The resultant mixture was extracted twice with 100 mL of dichloromethane each and the combined organic extracts were washed twice with 10 mL of phosphate buffer each, dried, and evaporated to dryness. The residue was dissolved in 60 mL of toluene, cooled to 0°C and extracted with three 50 mL portions of cold pH 9.5 carbonate buffer. The aqueous extracts were adjusted to pH 3 with phosphoric acid, and extracted with five 40 mL portions of chloroform which were combined, dried and evaporated to dryness. The residue was purified by column chromatography using 25% ethylacetate/hexane to afford 1.9 g of b-ketoester (39%).

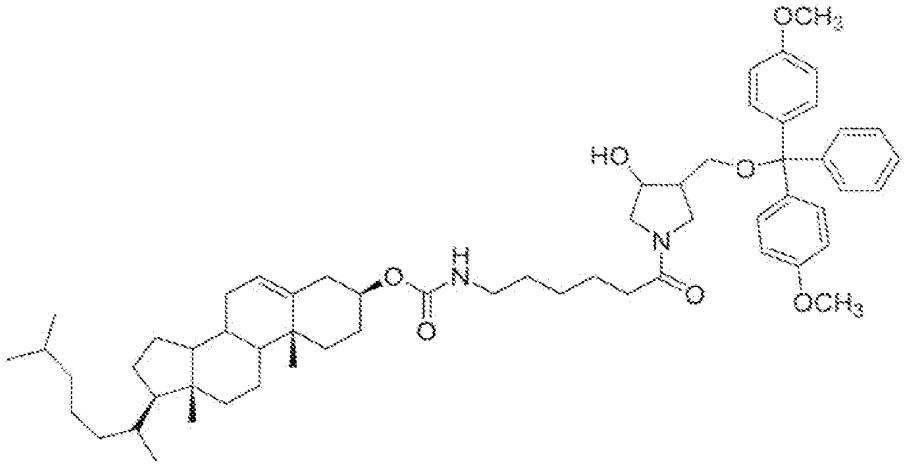
[6-(3-Hydroxy-4-hydroxymethyl-pyrrolidin-1-yl)-6-oxo-hexyl]-carbamic acid 17-(1,5-dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl ester **AF**



AF

Methanol (2 mL) was added dropwise over a period of 1 h to a refluxing mixture of b-ketoester AE (1.5 g, 2.2 mmol) and sodium borohydride (0.226 g, 6 mmol) in tetrahydrofuran (10 mL). Stirring was continued at reflux temperature for 1 h. After cooling to room temperature, 1 N HCl (12.5 mL) was added, the mixture was extracted with ethylacetate (3 x 40 mL). The combined ethylacetate layer was dried over anhydrous sodium sulfate and concentrated under vacuum to yield the product which was purified by column chromatography (10% MeOH/$CHCl_3$) (89%).

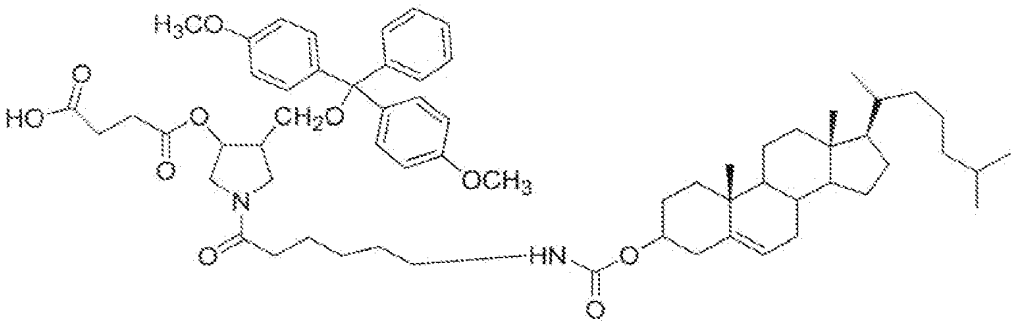
(6-{3-[Bis-(4-methoxy-phenyl)-phenyl-methoxymethyl]-4-hydroxy-pyrrolidin-1-yl}-6-oxo-hexyl)-carbamic acid 17-(1,5-dimethyl-hexyl)-10,13-dimethyl-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H-cyclopenta[a]phenanthren-3-yl ester **AG**



AG

Diol AF (1.25 gm 1.994 mmol) was dried by evaporating with pyridine (2 x 5 mL) *in vacuo*. Anhydrous pyridine (10 mL) and 4,4'-dimethoxytritylchloride (0.724 g, 2.13 mmol) were added with stirring. The reaction was carried out at room temperature overnight. The reaction was quenched by the addition of methanol. The reaction mixture was concentrated under vacuum and to the residue dichloromethane (50 mL) was added. The organic layer was washed with 1M aqueous sodium bicarbonate. The organic layer was dried over anhydrous sodium sulfate, filtered and concentrated. The residual pyridine was removed by evaporating with toluene. The crude product was purified by column chromatography (2% MeOH/Chloroform, Rf = 0.5 in 5% MeOH/$CHCl_3$) (1.75 g, 95%).

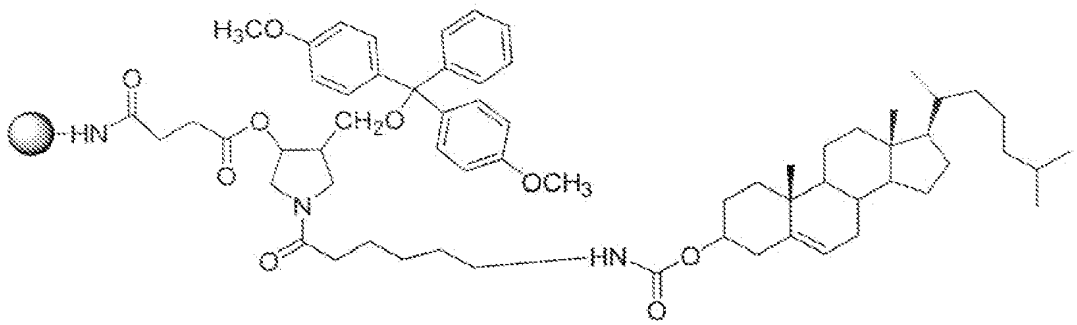
Succinic acid mono-(4-[bis-(4-methoxy-phenyl)-phenyl-methoxymethyl]-1-{6-[17-(1,5-dimethyl-hexyl)-10,13-dimethyl 2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-1H cyclopenta[a]phenanthren-3-yloxycarbonylamino}-hexanoyl}-pyrrolidin-3-yl) ester **AH**



AH

Compound AG (1.0 g, 1.05 mmol) was mixed with succinic anhydride (0.150 g, 1.5 mmol) and DMAP (0.073 g, 0.6 mmol) and dried in a vacuum at 40°C overnight. The mixture was dissolved in anhydrous dichloroethane (3 mL), triethylamine (0.318 g, 0.440 mL, 3.15 mmol) was added and the solution was stirred at room temperature under argon atmosphere for 16 h. It was then diluted with dichloromethane (40 mL) and washed with ice cold aqueous citric acid (5 wt%, 30 mL) and water (2 X 20 mL). The organic phase was dried over anhydrous sodium sulfate and concentrated to dryness. The residue was used as such for the next step.

Cholesterol derivatised CPG **AI**



AI

Succinate AH (0.254 g, 0.242 mmol) was dissolved in a mixture of dichloromethane/acetonitrile (3:2, 3 mL). To that solution DMAP (0.0296 g, 0.242

mmol) in acetonitrile (1.25 mL), 2,2'-Dithio-bis(5-nitropyridine) (0.075 g, 0.242 mmol) in acetonitrile/dichloroethane (3:1, 1.25 mL) were added successively. To the resulting solution triphenylphosphine (0.064 g, 0.242 mmol) in acetonitrile (0.6 ml) was added. The reaction mixture turned bright orange in color. The solution was agitated briefly using a wrist-action shaker (5 mins). Long chain alkyl amine-CPG (LCAA-CPG) (1.5 g, 61 mM) was added. The suspension was agitated for 2 h. The CPG was filtered through a sintered funnel and washed with acetonitrile, dichloromethane and ether successively. Unreacted amino groups were masked using acetic anhydride/pyridine. The achieved loading of the CPG was measured by taking UV measurement (37 mM/g).

The synthesis of siRNAs bearing a 5'-12-dodecanoic acid bisdecylamide group (herein referred to as "5'-C32-") or a 5'-cholesteryl derivative group (herein referred to as "5'-Chol-") was performed as described in WO 2004/065601, except that, for the cholesteryl derivative, the oxidation step was performed using the Beaucage reagent in order to introduce a phosphorothioate linkage at the 5'-end of the nucleic acid oligomer.

Nucleic acid sequences are represented below using standard nomenclature, and specifically the abbreviations of Table 4.

Table 4: Abbreviations of nucleotide monomers used in nucleic acid sequence representation. It will be understood that these monomers, when present in an oligonucleotide, are mutually linked by 5'-3'-phosphodiester bonds.

Abbreviation[a]	Nucleotide(s)
A, a	2'-deoxy-adenosine-5'-phosphate, adenosine-5'-phosphate
C, c	2'-deoxy-cytidine-5'-phosphate, cytidine-5'-phosphate
G, g	2'-deoxy-guanosine-5'-phosphate, guanosine-5'-phosphate
T, t	2'-deoxy-thymidine-5'-phosphate, thymidine-5'-phosphate
U, u	2'-deoxy-uridine-5'-phosphate, uridine-5'-phosphate
N, n	any 2'-deoxy-nucleotide/nucleotide (G, A, C, or T, g, a, c or u)
Am	*2'-O*-methyladenosine-5'-phosphate
Cm	*2'-O*-methylcytidine-5'-phosphate
Gm	*2'-O*-methylguanosine-5'-phosphate
Tm	*2'-O*-methyl-thymidine-5'-phosphate
Um	*2'-O*-methyluridine-5'-phosphate
Af	2'-fluoro-2'-deoxy-adenosine-5'-phosphate
Cf	2'-fluoro-2'-deoxy-cytidine-5'-phosphate
Gf	2'-fluoro-2'-deoxy-guanosine-5'-phosphate
Tf	2'-fluoro-2'-deoxy-thymidine-5'-phosphate
Uf	2'-fluoro-2'-deoxy-uridine-5'-phosphate
A, C, G, T, U, a, c, g, t, u	underlined: nucleoside-5'-phosphorothioate
am, cm, gm, tm, um	underlined: 2-*O*-methyl-nucleoside-5'-phosphorothioate

[a]capital letters represent 2'-deoxyribonucleotides (DNA), lower case letters represent ribonucleotides (RNA)

dsRNA expression vectors

In another aspect of the invention, Eg5 specific dsRNA molecules that modulate Eg5 gene expression activity are expressed from transcription units inserted into DNA or RNA vectors (see, e.g., Couture, A, et al., *TIG.* (1996), **12**:5-10; Skillern, A., et al., International PCT Publication No. WO 00/22113, Conrad, International PCT Publication No. WO 00/22114, and Conrad, US Pat. No.

6,054,299). These transgenes can be introduced as a linear construct, a circular plasmid, or a viral vector, which can be incorporated and inherited as a transgene integrated into the host genome. The transgene can also be constructed to permit it to be inherited as an extrachromosomal plasmid (Gassmann, et al., *Proc. Natl. Acad. Sci. USA* (1995) **92**:1292).

The individual strands of a dsRNA can be transcribed by promoters on two separate expression vectors and co-transfected into a target cell. Alternatively each individual strand of the dsRNA can be transcribed by promoters both of which are located on the same expression plasmid. In a preferred embodiment, a dsRNA is expressed as an inverted repeat joined by a linker polynucleotide sequence such that the dsRNA has a stem and loop structure.

The recombinant dsRNA expression vectors are generally DNA plasmids or viral vectors. dsRNA expressing viral vectors can be constructed based on, but not limited to, adeno-associated virus (for a review, see Muzyczka, et al., *Curr. Topics Micro. Immunol.* (1992) **158**:97-129)); adenovirus (see, for example, Berkner, et al., BioTechniques (1998) **6**:616), Rosenfeld et al. (1991, Science 252:431-434), and Rosenfeld et al. (1992), *Cell* 68:143-155)); or alphavirus as well as others known in the art. Retroviruses have been used to introduce a variety of genes into many different cell types, including epithelial cells, in vitro and/or in vivo (see, e.g., Eglitis, et al., *Science* (1985) **230**:1395-1398; Danos and Mulligan, *Proc. Natl. Acad. Sci. USA* (1998) **85**:6460-6464; Wilson et al., 1988, Proc. Natl. Acad. Sci. USA 85:3014-3018; Armentano et al., 1990, Proc. Natl. Acad. Sci. USA 87:61416145; Huber et al., 1991, Proc. Natl. Acad. Sci. USA 88:8039-8043; Ferry et al., 1991, Proc. Natl. Acad. Sci. USA 88:8377-8381; Chowdhury et al., 1991, Science 254:1802-1805; van Beusechem. et al., 1992, Proc. Nad. Acad. Sci. USA 89:7640-19 ; Kay et al., 1992, Human Gene Therapy 3:641-647; Dai et al., 1992, Proc. Natl.Acad. Sci. USA 89:10892-10895; Hwu et al., 1993, J. Immunol. 150:4104-4115; U.S. Patent No. 4,868,116; U.S. Patent No. 4,980,286; PCT Application WO 89/07136; PCT

Application WO 89/02468; PCT Application WO 89/05345; and PCT Application WO 92/07573). Recombinant retroviral vectors capable of transducing and expressing genes inserted into the genome of a cell can be produced by transfecting the recombinant retroviral genome into suitable packaging cell lines such as PA317 and Psi-CRIP (Comette et al., 1991, Human Gene Therapy 2:5-10; Cone et al., 1984, Proc. Natl. Acad. Sci. USA 81:6349). Recombinant adenoviral vectors can be used to infect a wide variety of cells and tissues in susceptible hosts (e.g., rat, hamster, dog, and chimpanzee) (Hsu et al., 1992, J. Infectious Disease, 166:769), and also have the advantage of not requiring mitotically active cells for infection.

The promoter driving dsRNA expression in either a DNA plasmid or viral vector of the invention may be a eukaryotic RNA polymerase I (e.g. ribosomal RNA promoter), RNA polymerase II (e.g. CMV early promoter or actin promoter or U1 snRNA promoter) or generally RNA polymerase III promoter (e.g. U6 snRNA or 7SK RNA promoter) or a prokaryotic promoter, for example the T7 promoter, provided the expression plasmid also encodes T7 RNA polymerase required for transcription from a T7 promoter. The promoter can also direct transgene expression to the pancreas (see, e.g. the insulin regulatory sequence for pancreas (Bucchini et al., 1986, Proc. Natl. Acad. Sci. USA 83:2511-2515)).

In addition, expression of the transgene can be precisely regulated, for example, by using an inducible regulatory sequence and expression systems such as a regulatory sequence that is sensitive to certain physiological regulators, e.g., circulating glucose levels, or hormones (Docherty et al., 1994, FASEB J. 8:20-24). Such inducible expression systems, suitable for the control of transgene expression in cells or in mammals include regulation by ecdysone, by estrogen, progesterone, tetracycline, chemical inducers of dimerization, and isopropyl-beta-D1 - thiogalactopyranoside (EPTG). A person skilled in the art would be able to choose the

appropriate regulatory/promoter sequence based on the intended use of the dsRNA transgene.

Generally, recombinant vectors capable of expressing dsRNA molecules are delivered as described below, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of dsRNA molecules. Such vectors can be repeatedly administered as necessary. Once expressed, the dsRNAs bind to target RNA and modulate its function or expression. Delivery of dsRNA expressing vectors can be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that allows for introduction into a desired target cell.

dsRNA expression DNA plasmids are typically transfected into target cells as a complex with cationic lipid carriers (e.g. Oligofectamine) or non-cationic lipid-based carriers (e.g. Transit-TKO^{TM}). Multiple lipid transfections for dsRNA-mediated knockdowns targeting different regions of a single Eg5 gene or multiple Eg5 genes over a period of a week or more are also contemplated by the invention. Successful introduction of the vectors of the invention into host cells can be monitored using various known methods. For example, transient transfection. can be signaled with a reporter, such as a fluorescent marker, such as Green Fluorescent Protein (GFP). Stable transfection. of ex vivo cells can be ensured using markers that provide the transfected cell with resistance to specific environmental factors (e.g., antibiotics and drugs), such as hygromycin B resistance.

The Eg5 specific dsRNA molecules can also be inserted into vectors and used as gene therapy vectors for human patients. Gene therapy vectors can be delivered to a subject by, for example, intravenous injection, local administration (see U.S. Patent 5,328,470) or by stereotactic injection (see e.g., Chen et al. (1994) Proc. Natl. Acad. Sci. USA 91:3054-3057). The pharmaceutical preparation of the gene therapy vector

can include the gene therapy vector in an acceptable diluent, or can comprise a slow release matrix in which the gene delivery vehicle is imbedded. Alternatively, where the complete gene delivery vector can be produced intact from recombinant cells, e.g., retroviral vectors, the pharmaceutical preparation can include one or more cells which produce the gene delivery system.

Eg5 siRNA in vitro screening via cell proliferation

As silencing of Eg5 has been shown to cause mitotic arrest (Weil, D, et al [2002] Biotechniques 33: 1244-8), a cell viability assay was used for siRNA activity screening. HeLa cells (14000 per well [Screens 1 and 3] or 10000 per well [Screen2])) were seeded in 96-well plates and simultaneously transfected with Lipofectamine 2000 (Invitrogen) at a final siRNA concentration in the well of 30 nM and at final concentrations of 50 nM (1st screen) and 25 nM (2nd screen). A subset of duplexes was tested at 25 nM in a third screen (Table 5).

Seventy-two hours post-transfection, cell proliferation was assayed the addition of WST-1 reagent (Roche) to the culture medium, and subsequent absorbance measurement at 450 nm. The absorbance value for control (non-transfected) cells was considered 100 percent, and absorbances for the siRNA transfected wells were compared to the control value. Assays were performed in sextuplicate for each of three screens. A subset of the siRNAs was further tested at a range of siRNA concentrations. Assays were performed in HeLa cells (14000 per well; method same as above, Table 5).

	Relative absorbance at 450 nm					
	Screen I		Screen II		Screen III	
Duplex	mean	sd	Mean	sd	mean	Sd
AL-DP-6226	20	10	28	11	43	9
AL-DP-6227	66	27	96	41	108	33
AL-DP-6228	56	28	76	22	78	18
AL-DP-6229	17	3	31	9	48	13
AL-DP-6230	48	8	75	11	73	7
AL-DP-6231	8	1	21	4	41	10
AL-DP-6232	16	2	37	7	52	14
AL-DP-6233	31	9	37	6	49	12
AL-DP-6234	103	40	141	29	164	45
AL-DP-6235	107	34	140	27	195	75
AL-DP-6236	48	12	54	12	56	12
AL-DP-6237	73	14	108	18	154	37
AL-DP-6238	64	9	103	10	105	24
AL-DP-6239	9	1	20	4	31	11
AL-DP-6240	99	7	139	16	194	43

AL-DP-6241	43	9	54	12	66	19
AL-DP-6242	6	1	15	7	36	8
AL-DP-6243	7	2	19	5	33	13
AL-DP-6244	7	2	19	3	37	13
AL-DP-6245	25	4	45	10	58	9
AL-DP-6246	34	8	65	10	66	13
AL-DP-6247	53	6	78	14	105	20
AL-DP-6248	7	0	22	7	39	12
AL-DP-6249	36	8	48	13	61	7

Table 5

The nine siRNA duplexes that showed the greatest growth inhibition in Table 5 were re-tested at a range of siRNA concentrations in HeLa cells. The siRNA concentrations tested were 100 nM, 33.3 nM, 11.1 nM, 3.70 nM, 1.23 nM, 0.41 nM, 0.14 nM and 0.046 nM. Assays were performed in sextuplicate, and the concentration of each siRNA resulting in fifty percent inhibition of cell proliferation (IC_{50}) was calculated. This dose-response analysis was performed between two and four times for each duplex. Mean IC_{50} values (nM) are given in Table 6.

Duplex	Mean IC_{50}
AL-DP-6226	15.5
AL-DP-6229	3.4
AL-DP-6231	4.2
AL-DP-6232	17.5
AL-DP-6239	4.4
AL-DP-6242	5.2
AL-DP-6243	2.6
AL-DP-6244	8.3
AL-DP-6248	1.9

Table 6

Eg5 siRNA in vitro screening via cell proliferation

Directly before transfection, Hela S3 (ATCC-Number: CCL-2.2, LCG Promochem GmbH, Wesel, Germany) cells were seeded at 1.5 x 10^4 cells / well on 96-well plates (Greiner Bio-One GmbH, Frickenhausen, Germany) in 75 µl of growth medium (Ham's F12, 10% fetal calf serum, 100u penicillin / 100 µg/ml streptomycin, all from Biochrom AG, Berlin, Germany). Transfections were performed in quadruplicates. For each well 0.5 µl Lipofectamine2000 (Invitrogen GmbH, Karlsruhe, Germany) were mixed with 12 µl Opti-MEM (Invitrogen) and incubated for 15 min at room temperature. For the siRNA concentration being 50 nM in the 100

µl transfection volume, 1 µl of a 5 µM siRNA were mixed with 11.5 µl Opti-MEM per well, combined with the Lipofectamine2000-Opti-MEM mixture and again incubated for 15 minutes at room temperature. siRNA-Lipofectamine2000-complexes were applied completely (25 µl each per well) to the cells and cells were incubated for 24 h at 37°C and 5 % CO_2 in a humidified incubator (Heraeus GmbH, Hanau). The single dose screen was done once at 50 nM and at 25 nM, respectively.

Cells were harvested by applying 50 µl of lysis mixture (content of the QuantiGene bDNA-kit from Genospectra, Fremont, USA) to each well containing 100 µl of growth medium and were lysed at 53°C for 30 min. Afterwards, 50 µl of the lysates were incubated with probesets specific to human Eg5 and human GAPDH and proceeded according to the manufacturer's protocol for QuantiGene. In the end chemoluminescence was measured in a Victor2-Light (Perkin Elmer, Wiesbaden, Germany) as RLUs (relative light units) and values obtained with the hEg5 probeset were normalized to the respective GAPDH values for each well. Values obtained with siRNAs directed against Eg5 were related to the value obtained with an unspecific siRNA (directed against HCV) which was set to 100% (Tables 1, 2 and 3).

Effective siRNAs from the screen were further characterized by dose response curves. Transfections of dose response curves were performed at the following concentrations: 100 nM, 16.7 nM, 2.8 nM, 0.46 nM, 77 picoM, 12.8 picoM, 2.1 picoM, 0.35 picoM, 59.5 fM, 9.9 fM and mock (no siRNA) and diluted with Opti-MEM to a final concentration of 12.5 µl according to the above protocol. Data analysis was performed by using the Microsoft Excel add-in software XL-fit 4.2 (IDBS, Guildford, Surrey, UK) and applying the dose response model number 205 (Tables 1, 2 and 3).

The lead siRNA AD12115 was additionally analyzed by applying the WST-proliferation assay from Roche (as previously described).

A subset of 34 duplexes from Table 2 that showed greatest activity was assayed by transfection in HeLa cells at final concentrations ranging from 100nM to 10fM. Transfections were performed in quadruplicate. Two dose-response assays were performed for each duplex. The concentration giving 20% (IC20), 50% (IC50) and 80% (IC80) reduction of KSP mRNA was calculated for each duplex. (Table 7).

Concentrations given in pM

	IC20s		IC50s		IC80s	
Duplex name	**1st screen**	**2nd screen**	**1st screen**	**2nd screen**	**1st screen**	**2nd screen**
AD12077	1.19	0.80	6.14	10.16	38.63	76.16
AD12078	25.43	25.43	156.18	156.18	ND	ND
AD12085	9.08	1.24	40.57	8.52	257.68	81.26
AD12095	1.03	0.97	9.84	4.94	90.31	60.47
AD12113	4.00	5.94	17.18	28.14	490.83	441.30
AD12115	0.60	0.41	3.79	3.39	23.45	23.45
AD12125	31.21	22.02	184.28	166.15	896.85	1068.11
AD12134	2.59	5.51	17.87	22.00	116.36	107.03
AD12149	0.72	0.50	4.51	3.91	30.29	40.89
AD12151	0.53	6.84	4.27	10.72	22.88	43.01
AD12152	155.45	7.56	807.36	66.69	13165.27	ND
AD12157	0.30	26.23	14.60	92.08	14399.22	693.31
AD12166	0.20	0.93	3.71	3.86	46.28	20.59
AD12180	28.85	28.85	101.06	101.06	847.21	847.21
AD12185	2.60	0.42	15.55	13.91	109.80	120.63

AD12194	2.08	1.11	5.37	5.09	53.03	30.92
AD12211	5.27	4.52	11.73	18.93	26.74	191.07
AD12257	4.56	5.20	21.68	22.75	124.69	135.82
AD12280	2.37	4.53	6.89	20.23	64.80	104.82
AD12281	8.81	8.65	19.68	42.89	119.01	356.08
AD12282	7.71	456.42	20.09	558.00	ND	ND
AD12285	ND	1.28	57.30	7.31	261.79	42.53
AD12292	40.23	12.00	929.11	109.10	ND	ND
AD12252	0.02	18.63	6.35	68.24	138.09	404.91
AD12275	25.76	25.04	123.89	133.10	1054.54	776.25
AD12266	4.85	7.80	10.00	32.94	41.67	162.65
AD12267	1.39	1.21	12.00	4.67	283.03	51.12
AD12264	0.92	2.07	8.56	15.12	56.36	196.78
AD12268	2.29	3.67	22.16	25.64	258.27	150.84
AD12279	1.11	28.54	23.19	96.87	327.28	607.27
AD12256	7.20	33.52	46.49	138.04	775.54	1076.76
AD12259	2.16	8.31	8.96	40.12	50.05	219.42
AD12276	19.49	6.14	89.60	59.60	672.51	736.72
AD12321	4.67	4.91	24.88	19.43	139.50	89.49

(ND-not determined)

Table 7

Silencing of liver Eg5/KSP in juvenile rats following single-bolus administration of LNP01 formulated siRNA

From birth until approximately 23 days of age, Eg5/KSP expression can be detected in the growing rat liver. Target silencing with a formulated Eg5/KSP siRNA was evaluated in juvenile rats.

KSP Duplex Tested

Duplex ID	Target	Sense	Antisense
AD6248	VEGF	AccGAAGuGuuGuuuGuccTsT (SEQ ID NO:1238)	GGAcAAAcAAcACUUCGGUTsT (SEQ ID NO:1239)

Methods

Dosing of animals. Male, juvenile Sprague-Dawley rats (19 days old) were administered single doses of lipidoid ("LNP01") formulated siRNA via tail vein injection. Groups of ten animals received doses of 10 milligrams per kilogram (mg/kg) bodyweight of either AD6248 or an unspecific siRNA. Dose level refers to the amount of siRNA duplex administered in the formulation. A third group received phosphate-buffered saline. Animals were sacrificed two days after siRNA administration. Livers were dissected, flash frozen in liquid Nitrogen and pulverized into powders.

mRNA measurements. Levels of Eg5/KSP mRNA were measured in livers from all treatment groups. Samples of each liver powder (approximately ten milligrams) were homogenized in tissue lysis buffer containing proteinase K. Levels of Eg5/KSP and GAPDH mRNA were measured in triplicate for each sample using the Quantigene branched DNA assay (GenoSpectra). Mean values for Eg5/KSP were normalized to mean GAPDH values for each sample. Group means were determined and normalized to the PBS group for each experiment.

Statistical analysis. Significance was determined by ANOVA followed by the Tukey post-hoc test

Results

Data Summary

Mean values (±standard deviation) for Eg5/KSP mRNA are given. Statistical significance (p value) versus the PBS group is shown (ns, not significant [p>0.05]).

Experiment 1

		VEGF/GAPDH	p value
PBS		1.0±0.47	
AD6248	10 mg/kg	0.47±0.12	<0.001
unspec	10 mg/kg	1.0±0.26	ns

A statistically significant reduction in liver Eg5/KSP mRNA was obtained following treatment with formulated AD6248 at a dose of 10 mg/kg.

Silencing of rat liver VEGF following intravenous infusion of LNP01 formulated siRNA duplexes

A "lipidoid" formulation comprising an equimolar mixture of two siRNAs was administered to rats. One siRNA (AD3133) was directed towards VEGF. The other (AD12115) was directed towards Eg5/KSP. Since Eg5/KSP expression is nearly undetectable in the adult rat liver, only VEGF levels were measured following siRNA treatment.

siRNA duplexes administered

Duplex ID	Target	Sense	Antisense
AD12115	Eg5/KSP	ucGAGAAucuAAAcuAAcuTsT (SEQ ID NO:1240)	AGUuAGUUuAGAUUCUCGATsT (SEQ ID NO: 1241)
AD3133	VEGF	GcAcAuAGGAGAGAuGAGCUsU (SEQ ID NO:1242)	AAGCUcAUCUCUCCuAuGuGCusG (SEQ ID NO:1243)

Key: A,G,C,U-ribonucleotides; c,u-2'-O-Me ribonucleotides; s-phorphorothioate.

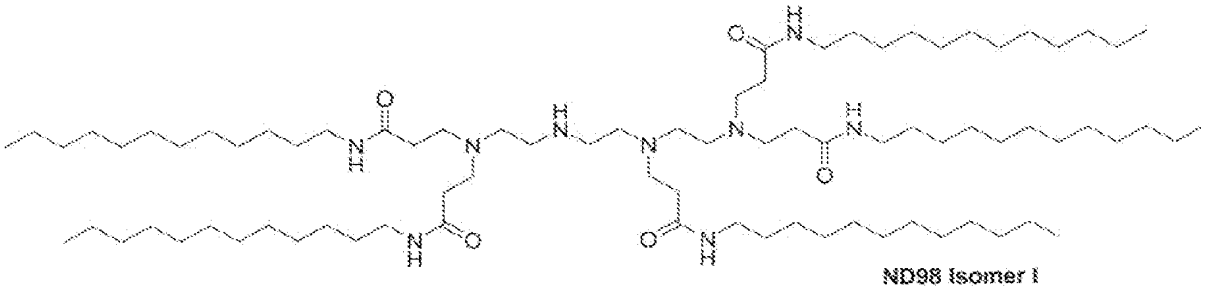
Methods

Dosing of animals. Adult, female Sprague-Dawley rats were administered lipidoid ("LNP01") formulated siRNA by a two-hour infusion into the femoral vein. Groups of four animals received doses of 5, 10 and 15 milligrams per kilogram (mg/kg) bodyweight of formulated siRNA. Dose level refers to the total amount of siRNA duplex administered in the formulation. A fourth group received phosphate-buffered saline. Animals were sacrificed 72 hours after the end of the siRNA infusion. Livers were dissected, flash frozen in liquid Nitrogen and pulverized into powders.

Formulation Procedure

The lipidoid ND98·4HCl (MW 1487) (Formula 1), Cholesterol (Sigma-Aldrich), and PEG-Ceramide C16 (Avanti Polar Lipids) were used to prepare lipid-siRNA nanoparticles. Stock solutions of each in ethanol were prepared: ND98, 133 mg/mL; Cholesterol, 25 mg/mL, PEG-Ceramide C16, 100 mg/mL. ND98, Cholesterol, and PEG-Ceramide C16 stock solutions were then combined in a 42:48:10 molar ratio. Combined lipid solution was mixed rapidly with aqueous siRNA (in sodium acetate pH 5) such that the final ethanol concentration was 35-45% and the final sodium acetate concentration was 100-300 mM. Lipid-siRNA nanoparticles formed spontaneously upon mixing. Depending on the desired particle

size distribution, the resultant nanoparticle mixture was in some cases extruded through a polycarbonate membrane (100 nm cut-off) using a thermobarrel extruder (Lipex Extruder, Northern Lipids, Inc). In other cases, the extrusion step was omitted. Ethanol removal and simultaneous buffer exchange was accomplished by either dialysis or tangential flow filtration. Buffer was exchanged to phosphate buffered saline (PBS) pH 7.2.



Formula I

Characterization of formulations

Formulations prepared by either the standard or extrusion-free method are characterized in a similar manner. Formulations are first characterized by visual inspection. They should be whitish translucent solutions free from aggregates or sediment. Particle size and particle size distribution of lipid-nanoparticles are measured by dynamic light scattering using a Malvern Zetasizer Nano ZS (Malvern, USA). Particles should be 20-300 nm, and ideally, 40-100 nm in size. The particle size distribution should be unimodal. The total siRNA concentration in the formulation, as well as the entrapped fraction, is estimated using a dye exclusion assay. A sample of the formulated siRNA is incubated with the RNA-binding dye Ribogreen (Molecular Probes) in the presence or absence of a formulation disrupting surfactant, 0.5% Triton-X100. The total siRNA in the formulation is determined by the signal from the sample containing the surfactant, relative to a standard curve. The

entrapped fraction is determined by subtracting the "free" siRNA content (as measured by the signal in the absence of surfactant) from the total siRNA content. Percent entrapped siRNA is typically >85%.

mRNA measurements. Samples of each liver powder (approximately ten milligrams) were homogenized in tissue lysis buffer containing proteinase K. Levels of VEGF and GAPDH mRNA were measured in triplicate for each sample using the Quantigene branched DNA assay (GenoSpectra). Mean values for VEGF were normalized to mean GAPDH values for each sample. Group means were determined and normalized to the PBS group for each experiment.

Protein measurements. Samples of each liver powder (approximately 60 milligrams) were homogenized in 1 ml RIPA buffer. Total protein concentrations were determined using the Micro BCA protein assay kit (Pierce). Samples of total protein from each animal was used to determine VEGF protein levels using a VEGF ELISA assay (R&D systems). Group means were determined and normalized to the PBS group for each experiment.

Statistical analysis. Significance was determined by ANOVA followed by the Tukey post-hoc test

Results

Data Summary

Mean values (±standard deviation) for mRNA (VEGF/GAPDH) and protein (rel. VEGF) are shown for each treatment group. Statistical significance (p value) versus the PBS group for each experiment is shown.

	VEGF/GAPDH	p value	rel VEGF	p value
PBS	1.0±0.17		1.0±0.17	

5 mg/kg	0.74±0.12	<0.05	0.23±0.03	<0.001
10 mg/kg	0.65±0.12	<0.005	0.22±0.03	<0.001
15 mg/kg	0.49±0.17	<0.001	0.20±0.04	<0.001

Statistically significant reductions in liver VEGF mRNA and protein were measured at all three siRNA dose levels.

TABLE 1

position in human access. #	sequence of total 23mer target site	SEQ ID	sense sequence (5'-3')	SEQ ID	antisense sequence (5'-3')	duplex name	single dose screen @ 25 nM [% residual mRNA]	SDs 2nd screen (among quadruplicates)
385-407	ACCGAAGUGUUGUUUGUCCAAUU	1	cGAAGuGuuGuuuGuccAATsT	2	UUGGAcAAAcAAcACUUCGTsT	AL-DP-6226	23%	3%
347-369	GAUGGUGUUUGGAGCAUCUACUA	3	uGGuGuuuGGAGcAucuAcTsT	4	GuAGAUGCUCcAAAcACcATsT	AL-DP-6227	69%	10%
1078-1100	AAUCUAAACUAACUAGAAUCCUC	5	ucuAAAcuAAcuAGAAuccTsT	6	GGAUUCuAGUuAGUUuAGATsT	AL-DP-6228	33%	2%
1067-1089	UCCUUAUCGAGAAUCUAAACUAA	7	cuuAucGAGAAucuAAAcuTsT	8	AGUUuAGAUUCUCGAuAAGTsT	AL-DP-6229	2%	2%
374-396	GAUUGAUGUUUACCGAAGUGUUG	9	uuGAuGuuuAccGAAGuGuTsT	10	AcACUUCGGuAAAcAUcAATsT	AL-DP-6230	66%	11%
205-227	UGGUGAGAUGCAGACCAUUUAAU	11	GuGAGAuGcAGAccAuuuATsT	12	uAAAUGGUCUGcAUCUcACTsT	AL-DP-6231	17%	1%
1176-1198	ACUCUGAGUACAUUGGAAUAUGC	13	ucuGAGuAcAuuGGAAuAuTsT	14	AuAUUCcAAUGuACUcAGATsT	AL-DP-6232	9%	3%
386-408	CCGAAGUGUUGUUUGUCCAAUUC	15	GAAGuGuuGuuuGuccAAuTsT	16	AUUGGAcAAAcAAcACUUCTsT	AL-DP-6233	24%	6%
416-438	AGUUAUUAUGGGCUAUAAUUGCA	17	uuAuuAuGGGcuAuAAuuGTsT	18	cAAUuAuAGCCcAuAAuAATsT	AL-DP-6234	91%	2%
485-507	GGAAGGUGAAAGGUCACCUAAUG	19	AAGGuGAAAGGucAccuAATsT	20	UuAGGUGACCUUUcACCUUTsT	AL-DP-6235	112%	4%
476-498	UUUUACAAUGGAAGGUGAAAGGU	21	uuAcAAuGGAAGGuGAAAGTsT	22	CUUUcACCUUCcAUUGuAATsT	AL-DP-6236	69%	4%
486-508	GAAGGUGAAAGGUCACCUAAUGA	23	AGGuGAAAGGucAccuAAuTsT	24	AUuAGGUGACCUUUcACCUTsT	AL-DP-6237	42%	2%
487-509	AAGGUGAAAGGUCACCUAAUGAA	25	GGuGAAAGGucAccuAAuGTsT	26	cAUuAGGUGACCUUUcACCTsT	AL-DP-6238	45%	2%
1066-1088	UUCCUUAUCGAGAAUCUAAACUA	27	ccuuAucGAGAAucuAAAcTsT	28	GUUuAGAUUCUCGAuAAGGTsT	AL-DP-6239	2%	1%
1256-1278	AGCUCUUAUUAAGGAGUAUACGG	29	cucuuAuuAAGGAGuAuAcTsT	30	GuAuACUCCUuAAuAAGAGTsT	AL-DP-6240	49%	2%
2329-2351	CAGAGAGAUUCUGUGCUUUGGAG	31	GAGAGAuucuGuGcuuuGGTsT	32	CcAAAGcAcAGAAUCUCUCTsT	AL-DP-6241	41%	2%
1077-1099	GAAUCUAAACUAACUAGAAUCCU	33	AucuAAAcuAAcuAGAAucTsT	34	GAUUCuAGUuAGUUuAGAUTsT	AL-DP-6242	8%	2%
1244-1266	ACUCACCAAAAAAGCUCUUAUUA	35	ucAccAAAAAAGcucuuAuTsT	36	AuAAGAGCUUUUUUGGUGATsT	AL-DP-6243	7%	1%
637-659	AAGAGCUUUUUGAUCUUCUUAAU	37	GAGcuuuuuGAucuucuuATsT	38	uAAGAAGAUcAAAAAGCUCTsT	AL-DP-6244	6%	2%
1117-1139	GGCGUACAAGAACAUCUAUAAUU	39	cGuAcAAGAAcAucuAuAATsT	40	UuAuAGAUGUUCUUGuACGTsT	AL-DP-6245	12%	2%
373-395	AGAUUGAUGUUUACCGAAGUGUU	41	AuuGAuGuuuAccGAAGuGTsT	42	cACUUCGGuAAAcAUcAAUTsT	AL-DP-6246	28%	3%
1079-1101	AUCUAAACUAACUAGAAUCCUCC	43	cuAAAcuAAcuAGAAuccuTsT	44	AGGAUUCuAGUuAGUUuAGTsT	AL-DP-6247	71%	4%
383-405	UUACCGAAGUGUUGUUUGUCCAA	45	AccGAAGuGuuGuuuGuccTsT	46	GGAcAAAcAAcACUUCGGUTsT	AL-DP-6248	5%	2%
200-222	CGUGGUGGUGAGAUGCAGACCAU	47	uGGuGGuGAGAuGcAGAccTsT	48	GGUCUGcAUCUcACcACcATsT	AL-DP-6249	28%	3%

TABLE 2

position in human access. #	sequence of total 19mer target site	seqID	sense sequence (5'-3')	seqID	antisense sequence (5'-3')	duplex name	1st single dose screen @ 50 nM [% residual mRNA]	SDs 1st screen (among quadruplicates)	2nd single dose screen @ 25 nM [% residual mRNA]	SDs 2nd screen (among quadruplicates)	3rd single dose screen @ 25 nM	SDs 3rd screen (among quadruplicates)
829-847	CAUACUCUAGUCGUUCCCA	49	cAuAcucuAGuCGuuccCATsT	50	UGGGAACGACuAGAGuAUGTsT	AD-12072	65%	2%	82%	5%		
246-264	AGCGCCCAUUCAAUAGUAG	51	AGcGcccAuucAAuAGuAGTsT	52	CuACuAUUGAAUGGGCGCUTsT	AD-12073	84%	1%	61%	6%		
238-256	GGAAAGCUAGCGCCCAUUC	53	GGAAAGcuAGcGcccAuucTsT	54	GAAUGGGCGCuAGCUUUCCTsT	AD-12074	51%	3%	36%	9%		
239-257	GAAAGCUAGCGCCCAUUCA	55	GAAAGcuAGcGcccAuucATsT	56	UGAAUGGGCGCuAGCUUUCTsT	AD-12075	56%	4%	36%	4%		
878-896	AGAAACUACGAUUGAUGGA	57	AGAAAcuAcGAuuGAuGGATsT	58	UCcAUcAAUCGuAGUUUCUTsT	AD-12076	21%	4%	13%	3%		
1064-1082	UGUUCCUUAUCGAGAAUCU	59	uGuuccuuAucGAGAAucuTsT	60	AGAUUCUCGAuAAGGAAcATsT	AD-12077	11%	2%	6%	1%		
3278-3296	CAGAUUACCUCUGCGAGCC	61	cAGAuuAccucuGcGAGccTsT	62	GGCUCGcAGAGGuAAUCUGTsT	AD-12078	23%	3%	9%	2%		
247-265	GCGCCCAUUCAAUAGUAGA	63	GcGcccAuucAAuAGuAGATsT	64	UCuACuAUUGAAUGGGCGCTsT	AD-12079	22%	10%	15%	7%		
434-452	UUGCACUAUCUUUGCGUAU	65	uuGcAcuAucuuuGcGuAuTsT	66	AuACGCAAAGAuAGUGcAATsT	AD-12080	68%	4%	52%	13%		
232-250	CAGAGCGGAAAGCUAGCGC	67	cAGAGcGGAAAGcuAGcGcTsT	68	GCGCuAGCUUUCCGCUCUGTsT	AD-12081	34%	5%	35%	24%		
1831-1849	AGACCUUAUUUGGUAAUCU	69	AGAccuuAuuuGGuAAucuTsT	70	AGAUuACcAAAuAAGGUCUTsT	AD-12082	20%	2%	92%	5%		
1105-1123	AUUCUCUUCUAGGGCGUAC	71	AuucucuucuAGGGcGuAcTsT	72	GuACGCCCuAGAAGAGAAUTsT	AD-12083	85%	6%	63%	10%		
536-554	GGCUGGUAUAAUUCCACGU	73	GGcuGGuAuAAuuccAcGuTsT	74	ACGUGGAAUuAuACcAGCCTsT	AD-12084	18%	6%	17%	4%		
236-254	GCGGAAAGCUAGCGCCCAU	75	GcGGAAAGcuAGcGcccAuTsT	76	AUGGGCGCuAGCUUUCCGCTsT	AD-12085	13%	4%	12%	4%		
435-453	UGCACUAUCUUUGCGUAUG	77	uGcAcuAucuuuGcGuAuGTsT	78	CAuACGcAAAGAuAGUGcATsT	AD-12086	26%	5%	17%	3%		
541-559	GUAUAAUUCCACGUACCCU	79	GuAuAAuuccAcGuAcccuTsT	80	AGGGuACGUGGAAUuAuACTsT	AD-12087	95%	4%	80%	6%		
1076-1094	AGAAUCUAAACUAACUAGA	81	AGAAucuAAAcuAAcuAGATsT	82	UCuAGUuAGUUuAGAUUCUTsT	AD-12088	29%	6%	29%	2%		
1432-1450	ACGAGCUGAAUAGGGUUAC	83	AcGAGcuGAAuAGGGuuAcTsT	84	GuAACCCuAUUcAGCUCGUTsT	AD-12089	69%	5%	64%	7%		
1821-1839	GAAGUACAUAAGACCUUAU	85	GAAGuAcAuAAGAccuuAuTsT	86	AuAAGGUCUuAUGuACUUCTsT	AD-12090	46%	15%	34%	5%		
2126-2144	GACAGUGCCGAUAAGAGUA	87	GAcAGuGccGAuAAGAGuATsT	88	uACUCuUAUCGGcACUGUCTsT	AD-12091	16%	6%	17%	3%		
2373-2391	AAACCACUUAGUAGUGUCC	89	AAAccAcuuAGuAGuGuccTsT	90	GGAcACuACuAAGUGGUUUTsT	AD-12092	82%	26%	63%	5%		
4026-4044	UCCCUAGACUUCCCUAUUU	91	ucccuAGAcuucccuAuuuTsT	92	AAAuAGGGAAGUCuAGGGATsT	AD-12093	89%	4%	70%	4%		
4030-4048	UAGACUUCCCUAUUUCGCU	93	uAGAcuucccuAuuucGcuTsT	94	AGCGAAAuAGGGAAGUCuATsT	AD-12094	46%	3%	34%	1%		
144-162	GCGUCGCAGCCAAAUUCGU	95	GcGucGcAGccAAAuucGuTsT	96	ACGAAUUUGGCUGCGACGCTsT	AD-12095	14%	2%	13%	2%		
242-260	AGCUAGCGCCCAUUCAAUA	97	AGcuAGcGcccAuucAAuATsT	98	uAUUGAAUGGGCGCuAGCUTsT	AD-12096	26%	11%	17%	1%		
879-897	GAAACUACGAUUGAUGGAG	99	GAAAcuAcGAuuGAuGGAGTsT	100	CUCcAUcAAUCGuAGUUUCTsT	AD-12097	23%	2%	21%	1%		
2134-2152	CCGAUAAGAUAGAAGAUCA	101	ccGAuAAGAuAGAAGAucATsT	102	UGAUCUUCuAUCUuAUCGGTsT	AD-12098	43%	14%	17%	3%		
245-263	UAGCGCCCAUUCAAUAGUA	103	uAGcGcccAuucAAuAGuATsT	104	uACuAUUGAAUGGGCGCuATsT	AD-12099	57%	2%	46%	6%		
444-462	UUUGCGUAUGGCCAAACUG	105	uuuGcGuAuGGccAAAcuGTsT	106	CAGUUUGGCcAuACGcAAATsT	AD-12100	101%	11%	98%	8%		
550-568	CACGUACCCUUCAUCAAAU	107	cAcGuAcccuucAucAAAuTsT	108	AUUUGAUGAAGGGuACGUGTsT	AD-12101	46%	7%	32%	2%		
442-460	UCUUUGCGUAUGGCCAAAC	109	ucuuuGcGuAuGGccAAAcTsT	110	GUUUGGCcAuACGcAAAGATsT	AD-12102	96%	17%	88%	18%		
386-404	CCGAAGUUCUGUUGUUCCA	111	ccGAAGuucuGuuGuuccATsT	112	UGGAAcAAcAGAACUUCGGTsT	AD-12103	19%	5%	20%	2%		

TABLE 2

233-251	AGAGCGGAAAGCUAGCGCC	113	AGAGcGGAAAGcuAGcGccTsT	114	GGCGCuAGCUUUCCGCUCUTsT	AD-12104	40%	6%	24%	2%		
243-261	GCUAGCGCCCAUUCAAUAG	115	GcuAGcGcccAuucAAuAGTsT	116	CuAUUGAAUGGGCGCuAGCTsT	AD-12105	39%	2%	36%	10%		
286-304	AAGUUAGUGUACGAACUGG	117	AAGuuAGuGuAcGAAcuGGTsT	118	CcAGUUCGuAcACuAACUUTsT	AD-12106	87%	6%	79%	19%		
294-312	GUACGAACUGGAGGAUUGG	119	GuAcGAAcuGGAGGAuuGGTsT	120	CcAAUCCUCcAGUUCGuACTsT	AD-12107	29%	2%	32%	16%		
296-314	ACGAACUGGAGGAUUGGCU	121	AcGAAcuGGAGGAuuGGcuTsT	122	AGCcAAUCCUCcAGUUCGUTsT	AD-12108	38%	4%	33%	8%		
373-391	AGAUUGAUGUUUACCGAAG	123	AGAuuGAuGuuuAccGAAGTsT	124	CUUCGGuAAAcAUcAAUCUTsT	AD-12109	49%	3%	44%	10%		
422-440	UAUGGCUAUAAUUGCACUU	125	uAuGGcuAuAAuuGcAcuuTsT	126	AAGUGcAAUuAuAGCcAuATsT	AD-12110	85%	5%	80%	24%		
441-459	AUCUUUGCGUAUGGCCAAA	127	AucuuuGcGuAuGGccAAATsT	128	UUUGGCcAuACGcAAAGAUTsT	AD-12111	64%	6%	71%	18%		
832-850	ACUCUAGUUGUUUCCACUC	129	AcucuAGuuGuuuccAcucTsT	130	GAGUGGAAACAACuAGAGUTsT	AD-12112	48%	4%	42%	5%		
881-899	AACUACGAUUGAUGGAGAA	131	AAcuAcGAuuGAuGGAGAATsT	132	UUCUCcAUcAAUCGuAGUUTsT	AD-12113	13%	0%	14%	3%		
975-993	GAUAAGAGAGCUCCGGAAG	133	GAuAAGAGAGcuccGGAAGTsT	134	CUUCCGGAGCUCUCUuAUCTsT	AD-12114	32%	6%	26%	4%		
1073-1091	UCGAGAAUCUAAACUAACU	135	ucGAGAAucuAAAcuAAcuTsT	136	AGUuAGUUuAGAUUCUCGATsT	AD-12115	8%	4%	7%	5%		
1084-1102	AACUAACUAGAAUCCUCCA	137	AAcuAAcuAGAAuccuccATsT	138	UGGAGGAUUCuAGUuAGUUTsT	AD-12116	74%	5%	61%	7%		
1691-1709	GGAUCGUAAGAAGGCAGUU	139	GGAucGuAAGAAGGcAGuuTsT	140	AACUGCCUUCUuACGAUCCTsT	AD-12117	21%	4%	20%	2%		
1693-1711	AUCGUAAGAAGGCAGUUGA	141	AucGuAAGAAGGcAGuuGATsT	142	UcAACUGCCUUCUuACGAUTsT	AD-12118	44%	4%	42%	6%		
1702-1720	AGGCAGUUGACCAACACAA	143	AGGcAGuuGAccAAcAcAATsT	144	UUGUGUUGGUcAACUGCCUTsT	AD-12119	37%	4%	24%	3%		
2131-2149	UGGCCUAUAAGAUAGAAGA	145	uGGccuAuAAGAuAGAAGATsT	146	UCUUCuAUCUuAUAGGCcATsT	AD-12120	22%	2%	19%	4%		
2412-2430	UCUAAGCAUAUAGUCAACA	147	ucuAAGcAuAuAGucAAcATsT	148	UGUUGACuAuAUGCUuAGATsT	AD-12121	32%	1%	22%	2%		
2859-2877	ACUAAGCUUAAUUGCUUUC	149	AcuAAGcuuAAuuGcuuucTsT	150	GAAAGcAAUuAAGCUuAGUTsT	AD-12122	36%	16%	19%	5%		
3294-3312	GCCCAGAUCAACCUUUAAU	151	GcccAGAucAAccuuuAAuTsT	152	AUuAAAGGUUGAUCUGGGCTsT	AD-12123	28%	1%	16%			
223-241	UUAAUUUGGCAGAGCGGAA	153	uuAAuuuGGcAGAGcGGAATsT	154	UUCCGCUCUGCcAAAUuAATsT	AD-12124	28%	2%	26%			
1070-1088	UUAUCGAGAAUCUAAACUA	155	uuAucGAGAAucuAAAcuATsT	156	uAGUUuAGAUUCUCGAuAATsT	AD-12125	15%	1%	14%			
244-262	CUAGCGCCCAUUCAAUAGU	157	cuAGcGcccAuucAAuAGuTsT	158	ACuAUUGAAUGGGCGCuAGTsT	AD-12126	51%	22%	27%			
257-275	AAUAGUAGAAUGUGAUCCU	159	AAuAGuAGAAuGuGAuccuTsT	160	AGGAUcAcAUUCuACuAUUTsT	AD-12127	54%	4%	42%	9%		
277-295	UACGAAAAGAAGUUAGUGU	161	uAcGAAAAGAAGuuAGuGuTsT	162	AcACuAACUUCUUUUCGuATsT	AD-12128	23%	1%	20%	2%		
284-302	AGAAGUUAGUGUACGAACU	163	AGAAGuuAGuGuAcGAAcuTsT	164	AGUUCGuAcACuAACUUCUTsT	AD-12129	22%	3%	19%	3%		
366-384	ACUAAACAGAUUGAUGUUU	165	AcuAAAcAGAuuGAuGuuuTsT	166	AAAcAUcAAUCUGUUuAGUTsT	AD-12130	53%	6%	43%	7%		
443-461	CUUUGCGUAUGGCCAAACU	167	cuuuGcGuAuGGccAAAcuTsT	168	AGUUUGGCcAuACGcAAAGTsT	AD-12131	28%	5%	22%	3%		
504-522	AAUGAAGAGUAUACCUGGG	169	AAuGAAGAGuAuAccuGGGTsT	170	CCcAGGuAuACUCUUcAUUTsT	AD-12132	68%	2%	90%	18%		
543-561	AUAAUUCCACGUACCCUUC	171	AuAAuuccAcGuAcccuucTsT	172	GAAGGGuACGUGGAAUuAUTsT	AD-12133	34%	3%	26%	6%		
551-569	ACGUACCCUUCAUCAAAUU	173	AcGuAcccuucAucAAAuuTsT	174	AAUUUGAUGAAGGGuACGUTsT	AD-12134	18%	3%	14%	2%		
552-570	CGUACCCUUCAUCAAAUUU	175	cGuAcccuucAucAAAuuuTsT	176	AAAUUUGAUGAAGGGuACGTsT	AD-12135	50%	6%	37%	4%		
553-571	GUACCCUUCAUCAAAUUUU	177	GuAcccuucAucAAAuuuuTsT	178	AAAAUUUGAUGAAGGGuACTsT	AD-12136	42%	19%	22%	2%		
577-595	AACUUACUGAUAAUGGUAC	179	AAcuuAcuGAuAAuGGuAcTsT	180	GuACcAUuAUcAGuAAGUUTsT	AD-12137	85%	12%	90%	4%		
602-620	UUCAGUCAAAGUGUCUCUG	181	uucAGucAAAGuGucucuGTsT	182	cAGAGAcACUUUGACUGAATsT	AD-12138	47%	6%	49%	1%		
652-670	UUCUUAAUCCAUCAUCUGA	183	uucuuAAuccAucAucuGATsT	184	UcAGAUGAUGGAUuAAGAATsT	AD-12139	80%	5%	72%	4%		
747-765	ACAGUACACAACAAGGAUG	185	AcAGuAcAcAAcAAGGAuGTsT	186	cAUCCUUGUUGUGuACUGUTsT	AD-12140	97%	22%	67%	9%		
877-895	AAGAAACUACGAUUGAUGG	187	AAGAAAcuAcGAuuGAuGGTsT	188	CcAUcAAUCGuAGUUUCUUTsT	AD-12141	120%	4%	107%	20%		
880-898	AAACUACGAUUGAUGGAGA	189	AAAcuAcGAuuGAuGGAGATsT	190	UCUCcAUcAAUCGuAGUUUTsT	AD-12142	55%	8%	33%	4%		
965-983	UGCAGCUGUUGAUAAGAGA	191	uGcAGcuGuuGAuAAGAGATsT	192	UCUCUuAUcAACAGCUGcATsT	AD-12143	64%	34%	19%	2%		

TABLE 2

1086-1104	CUAACUAGAAUCCUCCAGG	193	cuAAcuAGAAuccuccAGGTsT	194	CCUGGAGGAUUCuAGUuAGTsT	AD-12144	98%	29%	17%	2%		
1191-1209	GAAGAUGCUCAUAGAGCAA	195	GAAGAuGcucAuAGAGcAATsT	196	UUGCUCuAUGAGcAUCUUCTsT	AD-12145	27%	6%	19%	3%		
1195-1213	AUGCUCAUAGAGCAAAGAA	197	AuGcucAuAGAGcAAAGAATsT	198	UUCUUUGCUCuAUGAGcAUTsT	AD-12146	19%	20%	15%	1%		
1412-1430	AAAAAUUGGUGCUGUUGAG	199	AAAAAuuGGuGcuGuuGAGTsT	200	CUcAAcAGcACcAAUUUUUTsT	AD-12147	25%	9%	35%	3%		
1431-1449	GAGGAGCUGAAUAGGGUUA	201	GAGGAGcuGAAuAGGGuuATsT	202	uAACCCuAUUcAGCUCCUCTsT	AD-12148	38%	3%	56%	5%		
1433-1451	GGAGCUGAAUAGGGUUACA	203	GGAGcuGAAuAGGGuuAcATsT	204	UGuAACCCuAUUcAGCUCCTsT	AD-12149	8%	2%	12%	3%		
1434-1452	GAGCUGAAUAGGGUUACAG	205	GAGcuGAAuAGGGuuAcAGTsT	206	CUGuAACCCuAUUcAGCUCTsT	AD-12150	31%	2%	31%	7%		
1435-1453	AGCUGAAUAGGGUUACAGA	207	AGcuGAAuAGGGuuAcAGATsT	208	UCUGuAACCCuAUUcAGCUTsT	AD-12151	9%	5%	14%	2%		
1436-1454	GCUGAAUAGGGUUACAGAG	209	GcuGAAuAGGGuuAcAGAGTsT	210	CUCUGuAACCCuAUUcAGCTsT	AD-12152	3%	3%	23%	3%		
1684-1702	CCAAACUGGAUCGUAAGAA	211	ccAAAcuGGAucGuAAGAATsT	212	UUCUuACGAUCcAGUUUGGTsT	AD-12153	20%	6%	34%	4%		
1692-1710	GAUCGUAAGAAGGCAGUUG	213	GAucGuAAGAAGGcAGuuGTsT	214	cAACUGCCUUCUuACGAUCTsT	AD-12154	24%	7%	44%	3%		
1833-1851	ACCUUAUUUGGUAAUCUGC	215	AccuuAuuuGGuAAucuGcTsT	216	GcAGAUuACcAAAuAAGGUTsT	AD-12155	33%	6%	53%	11%		
1872-1890	UUAGAUACCAUUACUACAG	217	uuAGAuAccAuuAcuAcAGTsT	218	CUGuAGuAAUGGuAUCuAATsT	AD-12156	35%	9%	40%	5%		
1876-1894	AUACCAUUACUACAGUAGC	219	AuAccAuuAcuAcAGuAGcTsT	220	GCuACUGuAGuAAUGGuAUTsT	AD-12157	8%	3%	23%	4%		
1883-1901	UACUACAGUAGCACUUGGA	221	uAcuAcAGuAGcAcuuGGATsT	222	UCcAAGUGCuACUGuAGuATsT	AD-12158	13%	2%	22%	5%		
1987-2005	AAAGUAAAACUGUACUACA	223	AAAGuAAAAcuGuAcuAcATsT	224	UGuAGuAcAGUUUuACUUUTsT	AD-12159	34%	6%	46%	5%		
2022-2040	CUCAAGACUGAUCUUCUAA	225	cucAAGAcuGAucuucuAATsT	226	UuAGAAGAUcAGUCUUGAGTsT	AD-12160	19%	3%	31%	4%		
2124-2142	UUGACAGUGGCCGAUAAGA	227	uuGAcAGuGGccGAuAAGATsT	228	UCUuAUCGGCcACUGUcAATsT	AD-12161	68%	4%	83%	7%		
2125-2143	UGACAGUGGCCGAUAAGAU	229	uGAcAGuGGccGAuAAGAuTsT	230	AUCUuAUCGGCcACUGUcATsT	AD-12162	26%	7%	32%	7%		
2246-2264	GCAAGUUGGAAACCUAACU	231	GcAAGuuGGAAAccuAAcuTsT	232	AGUuAGGUUUCcAACUUGCTsT	AD-12163	55%	9%	40%	3%		
2376-2394	CCACUUAGUAGUGUCCAGG	233	ccAcuuAGuAGuGuccAGGTsT	234	CCUGGAcACuACuAAGUGGTsT	AD-12164			21%	3%		
2504-2522	AGAAGGUACAAAAUUGGUU	235	AGAAGGuAcAAAAuuGGuuTsT	236	AACcAAUUUUGuACCUUCUTsT	AD-12165	30%	3%	41%	4%		
2852-2870	UGGUUUGACUAAGCUUAAU	237	uGGuuuGAcuAAGcuuAAuTsT	238	AUuAAGCUuAGUcAAACcATsT	AD-12166	9%	10%	22%	9%		
2853-2871	GGUUUGACUAAGCUUAAUU	239	GGuuuGAcuAAGcuuAAuuTsT	240	AAUuAAGCUuAGUcAAACCTsT	AD-12167	26%	3%	30%	2%		
3110-3128	UCUAAGUCAAGAGCCAUCU	241	ucuAAGucAAGAGccAucuTsT	242	AGAUGGCUCUUGACUuAGATsT	AD-12168	54%	4%	59%	20%		
3764-3782	UCAUCCCUAUAGUUCACUU	243	ucAucccuAuAGuucAcuuTsT	244	AAGUGAACuAuAGGGAUGATsT	AD-12169	41%	4%	51%	16%		
3765-3783	CAUCCCUAUAGUUCACUUU	245	cAucccuAuAGuucAcuuuTsT	246	AAAGUGAACuAuAGGGAUGTsT	AD-12170	43%	4%	52%	20%		
4027-4045	CCCUAGACUUCCCUAUUUC	247	cccuAGAcuucccuAuuucTsT	248	GAAAuAGGGAAGUCuAGGGTsT	AD-12171	67%	3%	73%	25%		
4031-4049	AGACUUCCCUAUUUCGCUU	249	AGAcuucccuAuuucGcuuTsT	250	AAGCGAAAuAGGGAAGUCUTsT	AD-12172	53%	15%	37%	2%		
4082-4100	UCACCAAACCAUUUGUAGA	251	ucAccAAAccAuuuGuAGATsT	252	UCuACAAAUGGUUUGGUGATsT	AD-12173	39%	0%	39%	0%		
4272-4290	UCCUUUAAGAGGCCUAACU	253	uccuuuAAGAGGccuAAcuTsT	254	AGUuAGGCCUCUuAAAGGATsT	AD-12174	41%	5%	27%	0%		
4275-4293	UUUAAGAGGCCUAACUCAU	255	uuuAAGAGGccuAAcucAuTsT	256	AUGAGUuAGGCCUCUuAAATsT	AD-12175	28%	0%	38%	14%		
4276-4294	UUAAGAGGCCUAACUCAUU	257	uuAAGAGGccuAAcucAuuTsT	258	AAUGAGUuAGGCCUCUuAATsT	AD-12176	43%	3%	56%	25%		
4282-4300	GGCCUAACUCAUUCACCCU	259	GGccuAAcucAuucAcccuTsT	260	AGGGUGAAUGAGUuAGGCCTsT	AD-12177	68%	6%	74%	30%		
4571-4589	UGGUAUUUUUGAUCUGGCA	261	uGGuAuuuuuGAucuGGcATsT	262	UGCcAGAUcAAAAAuACcATsT	AD-12178	41%	4%	41%	6%		
4677-4695	AGUUUAGUGUGUAAAGUUU	263	AGuuuAGuGuGuAAAGuuuTsT	264	AAACUUuAcAcACuAAACUTsT	AD-12179	53%	5%	44%	5%		
152-170	GCCAAAUUCGUCUGCGAAG	265	GccAAAuucGucuGcGAAGTsT	266	CUUCGcAGACGAAUUUGGCTsT	AD-12180	16%	2%	13%	4%		
156-174	AAUUCGUCUGCGAAGAAGA	267	AAuucGucuGcGAAGAAGATsT	268	UCUUCUUCGcAGACGAAUUTsT	AD-12181	19%	3%	14%	2%		
491-509	UGAAAGGUCACCUAAUGAA	269	uGAAAGGucAccuAAuGAATsT	270	UUcAUuAGGUGACCUUUcATsT	AD-12182	26%	4%	28%	8%		
215-233	CAGACCAUUUAAUUUGGCA	271	cAGAccAuuuAAuuuGGcATsT	272	UGCcAAAUuAAAUGGUCUGTsT	AD-12183	26%	3%	19%	4%		

TABLE 2

216-234	AGACCAUUUAAUUGGCCAG	273	AGAccAuuuAAuuGGccAGTsT	274	CUGGcCAAUuAAAUGGUCUTsT	AD-12184	34%	2%	77%	3%		
416-434	AGUUAUUAUGGGCUAUAAU	275	AGuuAuuAuGGGcuAuAAuTsT	276	AUuAuAGCCCAuAAuAACUTsT	AD-12185	6%	1%	3%	1%		
537-555	GCUGGUAUAAUUCCACGUA	277	GcuGGuAuAAuuccAcGuATsT	278	uACGUGGAAUuAuACcAGCTsT	AD-12186	36%	3%	41%	6%		
221-239	AUUUAAUUGGCCAGAGCGG	279	AuuuAAuuGGccAGAGcGGTsT	280	CCGCUCUGGcCAAUuAAAUTsT	AD-12187	34%	17%	27%	1%		
222-240	UUUAAUUGGCCAGAGCGGA	281	uuuAAuuGGcAGAGcGGATsT	282	UCCGCUCUGGcCAAUuAAATsT	AD-12188	30%	3%	27%	4%		
227-245	UUGGCCAGAGCGGAAAGCU	283	uuGGccAGAGcGGAAAGcuTsT	284	AGCUUUCCGCUCUGGcCAATsT	AD-12189	82%	4%	48%	5%		
476-494	UUUUACAAUGGAAGGUGAA	285	uuuuACAAuGGAAGGuGAATsT	286	UUcACCUUCcAUUGuAAAATsT	AD-12190	33%	2%	36%	4%		
482-500	AAUGGAAGGUGAAAGGUCA	287	AAuGGAAGGuGAAAGGucATsT	288	UGACCUUUcACCUUCcAUUTsT	AD-12191	20%	2%	13%	5%		
208-226	UGAGAUGCAGACCAUUUAA	289	uGAGAuGcAGAccAuuuAATsT	290	UuAAAUGGUCUGcAUCUcATsT	AD-12192	21%	1%	23%	10%		
147-165	UCGCAGCCAAAUUCGUCUG	291	ucGcAGccAAAuucGucuGTsT	292	cAGACGAAUUUGGCUGCGATsT	AD-12193	64%	8%	98%	6%		
426-444	GGCUAUAAUUGCACUAUCU	293	GGcuAuAAuuGcAcuAucuTsT	294	AGAuAGUGcAAUuAuAGCCTsT	AD-12194	8%	2%	15%	4%		
2123-2141	AUUGACAGUGGCCGAUAAG	295	AuuGAcAGuGGccGAuAAGTsT	296	CUuAUCGGCcACUGUcAAUTsT	AD-12195	34%	2%	48%	3%		
4029-4047	CUAGACUUCCCUAUUUCGC	297	cuAGAcuucccuAuuucGcTsT	298	GCGAAAuAGGGAAGUCuAGTsT	AD-12196	34%	2%	51%	3%		
438-456	ACUAUCUUUGCGUAUGGCC	299	AcuAucuuuGcGuAuGGccTsT	300	GGCcAuACGcAAAGAuAGUTsT	AD-12197	75%	4%	93%	6%		
830-848	AUACUCUAGUCGUUCCCAC	301	AuAcucuAGucGuucccAcTsT	302	GUGGGAACGACuAGAGuAUTsT	AD-12198	55%	5%	48%	2%		
876-894	AAAGAAACUACGAUUCAUG	303	AAAGAAAcuACGAuucAuGTsT	304	cAUGAAUCGuAGUUUCUUUTsT	AD-12199	102%	6%	118%	9%		
115-133	GCCUUGAUUUUUUGGCGGG	305	GccuuGAuuuuuuGGcGGGTsT	306	CCCGCcAAAAAAUcAAGGCTsT	AD-12200	75%	5%	80%	10%		
248-266	CGCCCAUUCAAUAGUAGAA	307	cGcccAuucAAuAGuAGAATsT	308	UUCuACuAUUGAAUGGGCGTsT	AD-12201	42%	3%	16%	4%		
1834-1852	CCUUAUUUGGUAAUCUGCU	309	ccuuAuuuGGuAAucuGcuTsT	310	AGcAGAUuACcAAAuAAGGTsT	AD-12202	29%	4%	9%	3%		
3050-3068	AGAGACAAUUCCGGAUGUG	311	AGAGAcAAuuccGGAuGuGTsT	312	cAcAUCCGGAAUUGUCUCUTsT	AD-12203	114%	14%	83%	20%		
4705-4723	UGACUUUGAUAGCUAAAUU	313	uGAcuuuGAuAGcuAAAuuTsT	314	AAUUuAGCuAUcAAAGUcATsT	AD-12204	64%	7%	26%	5%		
229-247	UGGCAGAGCGGAAAGCUAG	315	uGGcAGAGcGGAAAGcuAGTsT	316	CuAGCUUUCCGCUCUGCcATsT	AD-12205	66%	12%	39%	4%		
234-252	GAGCGGAAAGCUAGCGCCC	317	GAGcGGAAAGcuAGcGcccTsT	318	GGGCGCuAGCUUUCCGCUCTsT	AD-12206	46%	3%	32%	12%		
282-300	AAAGAAGUUAGUGUACGAA	319	AAAGAAGuuAGuGuAcGAATsT	320	UUCGuAcACuAACUUCUUUTsT	AD-12207	57%	5%	40%	6%		
433-451	AUUGCACUAUCUUUGCGUA	321	AuuGcAcuAucuuuGcGuATsT	322	uACGcAAAGAuAGUGcAAUTsT	AD-12208	30%	8%	10%	5%		
540-558	GGUAUAAUUCCACGUACCC	323	GGuAuAAuuccAcGuAcccTsT	324	GGGuACGUGGAAUuAuACCTsT	AD-12209	101%	6%	102%	23%		
831-849	UACUCUAGUCGUUCCCACU	325	uAcucuAGucGuucccAcuTsT	326	AGUGGGAACGACuAGAGuATsT	AD-12210	38%	11%	27%	14%		
872-890	UAUGAAAGAAACUACGAUU	327	uAuGAAAGAAAcuAcGAuuTsT	328	AAUCGuAGUUUCUUUcAuATsT	AD-12211	16%	6%	10%	5%		
1815-1833	AUGCUAGAAGUACAUAAGA	329	AuGcuAGAAGuAcAuAAGATsT	330	UCUuAUGuACUUCuAGcAUTsT	AD-12212	59%	8%	65%	5%		
1822-1840	AAGUACAUAAGACCUUAUU	331	AAGuAcAuAAGAccuuAuuTsT	332	AAuAAGGUCUuAUGuACUUTsT	AD-12213	24%	2%	12%	2%		
3002-3020	ACAGCCUGAGCUGUUAAUG	333	AcAGccuGAGcuGuuAAuGTsT	334	cAUuAAcAGCUcAGGCUGUTsT	AD-12214	67%	14%	73%	12%		
3045-3063	AAAGAAGAGACAAUUCCGG	335	AAAGAAGAGAcAAuuccGGTsT	336	CCGGAAUUGUCUCUUCUUUTsT	AD-12215	29%	13%	13%	4%		
3224-3242	CACACUGGAGAGGUCUAAA	337	cAcAcuGGAGAGGucuAAATsT	338	UUuAGACCUCUCcAGUGUGTsT	AD-12216	36%	4%	13%	1%		
3226-3244	CACUGGAGAGGUCUAAAGU	339	cAcuGGAGAGGucuAAAGuTsT	340	ACUUuAGACCUCUCcAGUGTsT	AD-12217	36%	9%	11%	2%		
3227-3245	ACUGGAGAGGUCUAAAGUG	341	AcuGGAGAGGucuAAAGuGTsT	342	cACUUuAGACCUCUCcAGUTsT	AD-12218	35%	5%	17%	3%		
145-163	GGUCGCAGCCAAAUUCGUC	343	GGucGcAGccAAAuucGucTsT	344	GACGAAUUUGGCUGCGACCTsT	AD-12219	41%	9%	16%	1%		
1700-1718	GAAGGCAGUUGACCAACAC	345	GAAGGcAGuuGAccAAcAcTsT	346	GUGUUGGUcAACUGCCUUCTsT	AD-12220	37%	5%	23%	3%		
4291-4309	CAUUCACCCUGACAGAGUU	347	cAuucAcccuGAcAGAGuuTsT	348	AACUCUGUcAGGGUGAAUGTsT	AD-12221	58%	7%	39%	6%		
4278-4296	AAGAGCCUUAACUCAUUCA	349	AAGAGccuuAAcucAuucATsT	350	UGAAUGAGUuAAGGCUCUUTsT	AD-12222	74%	9%	53%	3%		
3051-3069	GAGACAAUUCCGGAUGUGG	351	GAGAcAAuuccGGAuGuGGTsT	352	CcAcAUCCGGAAUUGUCUCTsT	AD-12223	74%	10%	67%	7%		

TABLE 2

3058-3076	CUCCGGACUGGCAUGUAGA	353	cuccGGAcuGGcAuGuAGATsT	354	UCuAcAUGCcAGUCCGGAGTsT	AD-12224	24%	2%	11%	2%		
241-259	AAGCUAGCGCCCAUUCAAG	355	AAGcuAGcGcccAuucAAGTsT	356	CUUGAAUGGGCGCuAGCUUTsT	AD-12225	78%	5%	76%	14%		
285-303	GAAGUUAGUGUACGAACUG	357	GAAGuuAGuGuAcGAAcuGTsT	358	cAGUUCGuAcACuAACUUCTsT	AD-12226	45%	8%	48%	3%		
542-560	UAUAAUUCCACUUACCUUU	359	uAuAAuuccAcuuAccuuuTsT	360	AAAGGuAAGUGGAAUuAuATsT	AD-12227	61%	6%	47%	5%		
2127-2145	ACAGUGGCCGAUAAGAUAG	361	AcAGuGGccGAuAAGAuAGTsT	362	CuAUCUuAUCGGCcACUGUTsT	AD-12228	28%	3%	25%	5%		
3760-3778	GCUGUCAUCCCUAUAGUUC	363	GcuGucAucccuAuAGuucTsT	364	GAACuAuAGGGAUGAcAGCTsT	AD-12229	54%	13%	37%	6%		
3993-4011	GUCUUGCUAUGACUUGUGU	365	GucuuGcuAuGAcuuGuGuTsT	366	AcAcAAGUcAuAGcAAGAcTsT	AD-12230	70%	17%	65%	4%		
1696-1714	GUAAGAAGGCAGUUGACCA	367	GuAAGAAGGcAGuuGAccATsT	368	UGGUcAACUGCCUUCUuACTsT	AD-12231	32%	12%	22%	6%		
2122-2140	CAUUGACAGUGGCCGAUAA	369	cAuuGAcAGuGGccGAuAATsT	370	UuAUCGGCcACUGUcAAUGTsT	AD-12232	30%	3%	17%	2%		
2371-2389	AGAAACCACUUAGUAGUGU	371	AGAAAccAcuuAGuAGuGuTsT	372	AcACuACuAAGUGGUUUCUTsT	AD-12233	38%	2%	32%	3%		
3143-3161	GGAUUGUUCAUCAAUUGGC	373	GGAuuGuucAucAAuuGGcTsT	374	GCcAAUUGAUGAAcAAUCCTsT	AD-12234	90%	5%	35%	7%		
4277-4295	UAAGAGGCCUAACUCAUUC	375	uAAGAGGccuAAcucAuucTsT	376	GAAUGAGUuAGGCCUCUuATsT	AD-12235	57%	7%	46%	3%		
287-305	AGUUAGUGUACGAACUGGA	377	AGuuAGuGuAcGAAcuGGATsT	378	UCcAGUUCGuAcACuAACUTsT	AD-12236	34%	8%	16%	2%		
1823-1841	AGUACAUAAGACCUUAUUU	379	AGuAcAuAAGAccuuAuuuTsT	380	AAAuAAGGUCUuAUGuACUTsT	AD-12237	43%	9%	32%	8%		
3379-3397	UGAGCCUUGUGUAUAGAUU	381	uGAGccuuGuGuAuAGAuuTsT	382	AAUCuAuAcAcAAGGCUcATsT	AD-12238	42%	6%	34%	6%		
4273-4291	CCUUUAAGAGGCCUAACUC	383	ccuuuAAGAGGccuAAcucTsT	384	GAGUuAGGCCUCUuAAAGGTsT	AD-12239	42%	3%	40%	4%		
2375-2393	ACCACUUAGUAGUGUCCAG	385	AccAcuuAGuAGuGuccAGTsT	386	CUGGAcACuACuAAGUGGUTsT	AD-12240	47%	6%	36%	5%		
4439-4457	GAAACUUCCAAUUAUGUCU	387	GAAAcuuccAAuuAuGucuTsT	388	AGAcAuAAUUGGAAGUUUCTsT	AD-12241	69%	5%	70%	8%		
827-845	UGCAUACUCUAGUCGUUCC	389	uGcAuAcucuAGucGuuccTsT	390	GGAACGACuAGAGuAUGcATsT	AD-12242	61%	2%	47%	3%		
1699-1717	AGAAGGCAGUUGACCAACA	391	AGAAGGcAGuuGAccAAcATsT	392	UGUUGGUcAACUGCCUUCUTsT	AD-12243	28%	7%	15%	1%		
1824-1842	GUACAUAAGACCUUAUUUG	393	GuAcAuAAGAccuuAuuuGTsT	394	cAAAuAAGGUCUuAUGuACTsT	AD-12244	29%	6%	15%	1%		
429-447	UAUAAUUGCACUUACUUUG	395	uAuAAuuGcAcuuAcuuuGTsT	396	cAAAGuAAGUGcAAUuAuATsT	AD-12245	65%	6%	83%	13%		
656-674	UCUCUGUUACAAUACAUAU	397	ucucuGuuAcAAuAcAuAuTsT	398	AuAUGuAUUGuAAcAGAGATsT	AD-12246	29%	7%	31%	6%		
1194-1212	CAUGCUCAUAGAGCAAAGA	399	cAuGcucAuAGAGcAAAGATsT	400	UCUUUGCUCuAUGAGcAUGTsT	AD-12247	57%	11%	50%	3%		
392-410	UGUUGUUUGUCCAAUUCUG	401	uGuuGuuuGuccAAuucuGTsT	402	cAGAAUUGGAcAAAcAAcATsT	AD-12248	36%	8%	20%	3%	15%	7%
1085-1103	ACUAACUAGAAUCCUCCAG	403	AcuAAcuAGAAuccuccAGTsT	404	CUGGAGGAUUCuAGUuAGUTsT	AD-12249	44%	3%	70%	11%	103%	34%
2069-2087	UGUGGUGUCUACACUGAAA	405	uGuGGuGucuAcAcuGAAATsT	406	UUUcAGUGuAGAcACcAcATsT	AD-12250	47%	5%	18%	5%	17%	4%
4341-4359	UAUUAUGGGAGACCACCCA	407	uAuuAuGGGAGAccAcccATsT	408	UGGGUGGUCUCCcAuAAuATsT	AD-12251	101%	28%	35%	8%	60%	42%
759-777	AGGAUGAAGUUCUAUCAAA	409	AGGAuGAAGuucuAucAAATsT	410	UUUGAuAGAACUUcAUCCUTsT	AD-12252	94%	19%	8%	3%	5%	3%
973-991	UUGAUAAGAGAGCUCGGGA	411	uuGAuAAGAGAGcucGGGATsT	412	UCCCGAGCUCUCUuAUcAATsT	AD-12253	94%	33%	42%	6%	49%	27%
1063-1081	AUGUUCCUUAUCGAGAAUC	413	AuGuuccuuAucGAGAAucTsT	414	GAUUCUCGAuAAGGAAcAUTsT	AD-12254	101%	56%	70%	5%	80%	32%
1190-1208	GCAAUAUGCUCAUAGAGCA	415	GcAAuAuGcucAuAGAGcATsT	416	UGCUCuAUGAGcAuAUUGCTsT	AD-12255	163%	27%	26%	6%	36%	10%
1679-1697	CCAUUCCAAACUGGAUCGU	417	ccAuuccAAAcuGGAucGuTsT	418	ACGAUCcAGUUUGGAAUGGTsT	AD-12256	113%	62%	18%	3%	9%	4%
1703-1721	GGCAGUUGACCAACACAAU	419	GGcAGuuGAccAAcAcAAuTsT	420	AUUGUGUUGGUcAACUGCCTsT	AD-12257	10%	4%	9%	2%	6%	2%
1814-1832	CAUGCUAGAAGUACAUAAG	421	cAuGcuAGAAGuAcAuAAGTsT	422	CUuAUGuACUUCuAGcAUGTsT	AD-12258	27%	9%	18%	3%	20%	6%
1818-1836	CUAGAAGUACAUAAGACCU	423	cuAGAAGuAcAuAAGAccuTsT	424	AGGUCUuAUGuACUUCuAGTsT	AD-12259	20%	5%	10%	2%	13%	5%
1897-1915	UUGGAUCUUCUCACAUUAU	425	uuGGAucuucucAcAuuAuTsT	426	AuAAUGUGAGAAGAUCcAATsT	AD-12260	22%	7%	83%	7%	65%	13%
2066-2084	AACUGUGGUGUCUACACUG	427	AAcuGuGGuGucuAcAcuGTsT	428	cAGuGuAGAcACcAcAGUUTsT	AD-12261	122%	11%	66%	7%	80%	22%
2121-2139	UCAUUGACAGUGGCCGAUA	429	ucAuuGAcAGuGGccGAuATsT	430	uAUCGGCcACUGUcAAUGATsT	AD-12262	97%	30%	33%	6%	44%	18%
2280-2298	AUAAAGCAGACCCAUUCCC	431	AuAAAGcAGAcccAuucccTsT	432	GGGAAUGGGUCUGCUUuAUTsT	AD-12263	177%	57%	85%	11%	84%	15%

TABLE 2

2369-2387	ACAGAAACCACUUAGUAGU	433	AcAGAAAccAcuuAGuAGuTsT	434	ACuACuAAGUGGUUUCUGUTsT	AD-12264	37%	6%	10%	1%	10%	4%
2372-2390	GAAACCACUUAGUAGUGUC	435	GAAAccAcuuAGuAGuGucTsT	436	GAcACuACuAAGUGGUUUCTsT	AD-12265	40%	8%	17%	2%	20%	10%
2409-2427	AAAUCUAAGGAUAGUGUCA	437	AAAucuAAGGAuAGuGucATsT	438	UGAcACuAUCCUuAGAUUUTsT	AD-12266	33%	9%	8%	1%	6%	4%
2933-2951	UUAUUUAUACCCAUCAACA	439	uuAuuuAuAcccAucAAcATsT	440	UGUUGAUGGGuAuAAAuAATsT	AD-12267	34%	13%	11%	1%	6%	2%
3211-3229	ACAGAGGCAUUAACAGACU	441	AcAGAGGcAuuAAcAGAcuTsT	442	AGUCUGUuAAUGCCUCUGUTsT	AD-12268	34%	6%	11%	1%	9%	3%
3223-3241	ACACACUGGAGAGCUCCAA	443	AcAcAcuGGAGAGcuccAATsT	444	UuGGAGCUCUCCAGUGUGUTsT	AD-12269	54%	6%	33%	4%	29%	7%
3225-3243	ACACUGGAGAGCUCCAAAG	445	AcAcuGGAGAGcuccAAAGTsT	446	CUUuGGAGCUCUCCAGUGUTsT	AD-12270	52%	8%	29%	4%	27%	6%
3291-3309	CGAGCCCAGAUCAACCUUU	447	cGAGcccAGAucAAccuuuTsT	448	AAAGGUUGAUCUGGGCUCGTsT	AD-12271	53%	7%	27%	3%	19%	6%
4036-4054	UCCCUAUUUCGCUUUCUCC	449	ucccuAuuucGcuuucuccTsT	450	GGAGAAAGCGAAAuAGGGATsT	AD-12272	85%	15%	57%	7%	51%	16%
4180-4198	UCUAAAAUCACUGUCAACA	451	ucuAAAAucAcuGucAAcATsT	452	UGUUGAcAGUGAUUUuAGATsT	AD-12273	36%	6%	26%	2%	30%	5%
151-169	AGCCAAAUUCGUCUGCGAA	453	AGccAAAuucGucuGcGAATsT	454	UUCGcAGACGAAUUUGGCUTsT	AD-12274	75%	31%	40%	2%	50%	19%
250-268	CCCAUUCAAUAGUAGAAUG	455	cccAuucAAuAGuAGAAuGTsT	456	cAUUCuACuAUUGAAUGGGTsT	AD-12275	29%	9%	8%	2%	8%	4%
821-839	GAUGAAUGCAUACUCUAGU	457	GAuGAAuGcAuAcucuAGuTsT	458	ACuAGAGuAUGcAUUcAUCTsT	AD-12276	45%	19%	15%	2%	16%	12%
1060-1078	CUCAUGUUCCUUAUCGAGA	459	cucAuGuuccuuAucGAGATsT	460	UCUCGAuAAGGAAcAUGAGTsT	AD-12277	58%	17%	32%	2%	55%	14%
1075-1093	GAGAAUCUAAACUAACUAG	461	GAGAAucuAAAcuAAcuAGTsT	462	CuAGUuAGUUuAGAUUCUCTsT	AD-12278	120%	35%	96%	10%	124%	38%
1819-1837	UAGAAGUACAUAAGACCUU	463	uAGAAGuAcAuAAGAccuuTsT	464	AAGGUCUuAUGuACUUCuATsT	AD-12279	47%	29%	17%	1%	12%	4%
3003-3021	CAGCCUGAGCUGUUAAUGA	465	cAGccuGAGcuGuuAAuGATsT	466	UcAUuAAcAGCUcAGGCUGTsT	AD-12280	0%	0%	3%	1%		
3046-3064	AAGAAGAGACAAUUCCGGA	467	AAGAAGAGAcAAuuccGGATsT	468	UCCGGAAUUGUCUCUUCUUTsT	AD-12281	2%	0%	5%	2%		
3134-3152	UGCUGGUGUGGAUUGUUCA	469	uGcuGGuGuGGAuuGuucATsT	470	UGAAcAAUCcAcACcAGcATsT	AD-12282	3%	0%	25%	6%		
155-173	AAAUUCGUCUGCGAAGAAG	471	AAAuucGucuGcGAAGAAGTsT	472	CUUCUUCGcAGACGAAUUUTsT	AD-12283	3%	1%	35%	4%		
4596-4614	UUUCUGGAAGUUGAGAUGU	473	uuucuGGAAGuuGAGAuGuTsT	474	AcAUCUcAACUUCcAGAAATsT	AD-12284	9%	2%	49%	9%		
365-383	UACUAAACAGAUUGAUGUU	475	uAcuAAAcAGAuuGAuGuuTsT	476	AAcAUcAAUCUGUUuAGuATsT	AD-12285	7%	7%	21%	26%		
374-392	GAUUGAUGUUUACCGAAGU	477	GAuuGAuGuuuAccGAAGuTsT	478	ACUUCGGuAAAcAUcAAUCTsT	AD-12286	28%	34%	12%	7%		
436-454	GCACUAUCUUUGCGUAUGG	479	GcAcuAucuuuGcGuAuGGTsT	480	CcAuACGcAAAGAuAGUGCTsT	AD-12287	40%	21%	91%	23%		
539-557	UGGUAUAAUUCCACGUACC	481	uGGuAuAAuuccAcGuAccTsT	482	GGuACGUGGAAUuAuACcATsT	AD-12288	26%	7%	156%	146%		
1629-1647	AGCAAGCUGCUUAACACAG	483	AGcAAGcuGcuuAAcAcAGTsT	484	CUGUGUuAAGcAGCUUGCUTsT	AD-12289	43%	21%	220%	131%		
2370-2388	CAGAAACCACUUAGUAGUG	485	cAGAAAccAcuuAGuAGuGTsT	486	cACuACuAAGUGGUUUCUGTsT	AD-12290	3%	1%	81%	23%		
2676-2694	AACUUAUUGGAGGUUGUAA	487	AAcuuAuuGGAGGuuGuAATsT	488	UuAcAACCUCcAAuAAGUUTsT	AD-12291	4%	1%	70%	3%		
3228-3246	CUGGAGAGCUCCAAAGUGG	489	cuGGAGAGcuccAAAGuGGTsT	490	CcACUUuGGAGCUCUCcAGTsT	AD-12292	2%	1%	6%	2%		
3703-3721	AAAAAAGAUAUAAGGCAGU	491	AAAAAAGAuAuAAGGcAGuTsT	492	ACUGCCUuAuAUCUUUUUUTsT	AD-12293	4%	2%	36%	3%		
3737-3755	GAAUUUUGAUAUCUACCCA	493	GAAuuuuGAuAucuAcccATsT	494	UGGGuAGAuAUcAAAAUUCTsT	AD-12294	10%	6%	38%	3%		
4573-4591	GUAUUUUUGAUCUGGCAAC	495	GuAuuuuuGAucuGGcAAcTsT	496	GUUGCcAGAUcAAAAAuACTsT	AD-12295	29%	31%	37%	3%		
526-544	AGGAUCCCUUGGCUGGUAU	497	AGGAucccuuGGcuGGuAuTsT	498	AuACcAGCcAAGGGAUCCUTsT	AD-12296	82%	4%	83%	2%		
527-545	GGAUCCCUUGGCUGGUAUA	499	GGAucccuuGGcuGGuAuATsT	500	uAuACcAGCcAAGGGAUCCTsT	AD-12297	75%	3%	65%	2%		
256-274	CAAUAGUAGAAUGUGAUCC	501	cAAuAGuAGAAuGuGAuccTsT	502	GGAUcAcAUUCuACuAUUGTsT	AD-12298	73%	4%	60%	3%		
427-445	GCUAUAAUUGCACUAUCUU	503	GcuAuAAuuGcAcuAucuuTsT	504	AAGAuAGUGcAAUuAuAGCTsT	AD-12299	76%	4%	66%	4%		
554-572	UACCCUUCAUCAAAUUUUU	505	uAcccuucAucAAAuuuuuTsT	506	AAAAAUUUGAUGAAGGGuATsT	AD-12300	36%	4%	15%	1%		
1210-1228	AGAACAUAUUGAAUAAGCC	507	AGAAcAuAuuGAAuAAGccTsT	508	GGCUuAUUcAAuAUGUUCUTsT	AD-12301	33%	4%	18%	2%		
1414-1432	AAAUUGGUGCUGUUGAGGA	509	AAAuuGGuGcuGuuGAGGATsT	510	UCCUcAAcAGcACcAAUUUTsT	AD-12302	68%	8%	69%	3%		
1438-1456	UGAAUAGGGUUACAGAGUU	511	uGAAuAGGGuuAcAGAGuuTsT	512	AACUCUGuAACCCuAUUcATsT	AD-12303	35%	6%	17%	2%		

TABLE 2

1516-1534	AAGAACUUGAAACCACUCA	513	AAGAAcuuGAAAccAcucATsT	514	UGAGUGGUUUcAAGUUCUUTsT	AD-12304	70%	8%	70%	6%		
2279-2297	AAUAAAGCAGACCCAUUCC	515	AAuAAAGcAGAcccAuuccTsT	516	GGAAUGGGUCUGCUUuAUUTsT	AD-12305	63%	8%	80%	7%		
2939-2957	AUACCCAUCAACACUGGUA	517	AuAcccAucAAcAcuGGuATsT	518	uACcAGUGUUGAUGGGuAUTsT	AD-12306	23%	6%	20%	3%		
3142-3160	UGGAUUGUUCAUCAAUUGG	519	uGGAuuGuucAucAAuuGGTsT	520	CcAAUUGAUGAAcAAUCcATsT	AD-12307	78%	10%	58%	5%		
3229-3247	UGGAGAGGUCUAAAGUGGA	521	uGGAGAGGucuAAAGuGGATsT	522	UCcACUUuAGACCUCUCcATsT	AD-12308	27%	8%	25%	2%		
3763-3781	GUCAUCCCUAGAGUUCACU	523	GucAucccuAGAGuucAcuTsT	524	AGUGAACUCuAGGGAUGACTsT	AD-12309	58%	11%	42%	3%		
4801-4819	AUAAUGGCUAUAAUUUCUC	525	AuAAuGGcuAuAAuuucucTsT	526	GAGAAAUuAuAGCcAUuAUTsT	AD-12310	106%	23%	80%	2%		
529-547	AUCCCUUGGCUGGUAUAAU	527	AucccuuGGcuGGuAuAAuTsT	528	AUuAuACCAGCcAAGGGAUTsT	AD-12311	73%	12%	80%	2%		
425-443	GGGCUAUAAUUGCACUAUC	529	GGGcuAuAAuuGcAcuAucTsT	530	GAuAGUGcAAUuAuAGCCCTsT	AD-12312	39%	3%	36%	3%		
1104-1122	GAUUCUCUUGGAGGGCGUA	531	GAuucucuuGGAGGGcGuATsT	532	uACGCCCUCcAAGAGAAUCTsT	AD-12313	64%	9%	49%	6%		
1155-1173	GCAUCCCCCAAUCCUGAGG	533	GcAucccccAAuccuGAGGTsT	534	CCUcAGGAUUGGGGGAUGCTsT	AD-12314	28%	7%	24%	6%		
2403-2421	CAGCAGAAAUCUAAGGAUA	535	cAGcAGAAAucuAAGGAuATsT	536	uAUCCUuAGAUUUCUGCUGTsT	AD-12315	31%	7%	13%	3%		
3115-3133	GUCAAGAGCCAUCUGUAGA	537	GucAAGAGccAucuGuAGATsT	538	UCuAcAGAUGGCUCUUGACTsT	AD-12316	42%	5%	14%	2%		
3209-3227	AAACAGAGGCAUUAACACA	539	AAAcAGAGGcAuuAAcAcATsT	540	UGUGUuAAUGCCUCUGUUUTsT	AD-12317	34%	3%	19%	5%		
3293-3311	AGCCCAGAUCAACCUUUAA	541	AGcccAGAucAAccuuuAATsT	542	UuAAAGGUUGAUCUGGGCUTsT	AD-12318	46%	4%	25%	4%		
4574-4592	UAUUUUUGAUCUGGCAACC	543	uAuuuuuGAucuGGcAAccTsT	544	GGUUGCcAGAUcAAAAAuATsT	AD-12319	77%	3%	56%	4%		
352-370	UGUUUGGAGCAUCUACUAA	545	uGuuuGGAGcAucuAcuAATsT	546	UuAGuAGAUGCUCcAAAcATsT	AD-12320	55%	7%	41%	3%		
741-759	GAAAUUACAGUACACAACA	547	GAAAuuAcAGuAcAcAAcATsT	548	UGUUGUGuACUGuAAUUUCTsT	AD-12321	21%	3%	10%	2%		
1478-1496	ACUUGACCAGUGUAAAUCU	549	AcuuGAccAGuGuAAAucuTsT	550	AGAUUuAcACUGGUcAAGUTsT	AD-12322	27%	8%	30%	13%		
1483-1501	ACCAGUGUAAAUCUGACCU	551	AccAGuGuAAAucuGAccuTsT	552	AGGUcAGAUUuAcACUGGUTsT	AD-12323	26%	7%	35%	18%		
1967-1985	AGAACAAUCAUUAGCAGCA	553	AGAAcAAucAuuAGcAGcATsT	554	UGCUGcuAAUGAUUGUUCUTsT	AD-12324	27%	8%	27%	14%		
2247-2265	CAAUGUGGAAACCUAACUG	555	cAAuGuGGAAAccuAAcuGTsT	556	cAGUuAGGUUUCcAcAUUGTsT	AD-12325	32%	12%	32%	22%		
2500-2518	ACCAAGAAGGUACAAAAUU	557	AccAAGAAGGuAcAAAAuuTsT	558	AAUUUUGuACCUUCUUGGUTsT	AD-12326	42%	22%	45%	41%		
2508-2526	GGUACAAAAUUGGUUGAAG	559	GGuAcAAAAuuGGuuGAAGTsT	560	CUUcAACcAAUUUUGuACCTsT	AD-12327	36%	14%	37%	32%		
3138-3156	GGUGUGGAUUGUUCAUCAA	561	GGuGuGGAuuGuucAucAATsT	562	UUGAUGAAcAAUCcAcACCTsT	AD-12328	45%	2%	31%	3%		
4304-4322	AGAGUUCACAAAAAGCCCA	563	AGAGuucAcAAAAAGcccATsT	564	UGGGCUUUUUGUGAACUCUTsT	AD-12329	61%	4%	34%	3%		
4711-4729	UGAUAGCUAAAUUAAACCA	565	uGAuAGcuAAAuuAAAccATsT	566	UGGUUuAAUUuAGCuAUcATsT	AD-12330	63%	5%	38%	4%		
1221-1239	AAUAAGCCUGAAGUGAAUC	567	AAuAAGccuGAAGuGAAucTsT	568	GAUUcACUUcAGGCUuAUUTsT	AD-12331	50%	2%	26%	5%		
1705-1723	CAGUUGACCAACACAAUGC	569	cAGuuGAccAAcAcAAuGcTsT	570	GcAUUGUGUUGGUcAACUGTsT	AD-12332	80%	4%	53%	7%		
3137-3155	UGGUGUGGAUUGUUCAUCA	571	uGGuGuGGAuuGuucAucATsT	572	UGAUGAAcAAUCcAcACcATsT	AD-12333	34%	6%	12%	2%		
4292-4310	AUUCACCCUGACAGAGUUC	573	AuucAcccuGAcAGAGuucTsT	574	GAACUCUGUcAGGGUGAAUTsT	AD-12334	27%	2%	18%	3%		
1829-1847	UAAGACCUUAUUUGGUAAU	575	uAAGAccuuAuuuGGuAAuTsT	576	AUuACcAAAuAAGGUCUuATsT	AD-12335	54%	6%	60%	7%		
2244-2262	AAGCAAUGUGGAAACCUAA	577	AAGcAAuGuGGAAAccuAATsT	578	UuAGGUUUCcAcAUUGCUUTsT	AD-12336	45%	4%	36%	4%		
2888-2906	UCUGAAAUCUGAUACCCCA	579	ucuGAAAucuGAuAccccATsT	580	UGGGGuAUcAGAUUUcAGATsT	AD-12337	30%	7%	19%	2%		

TABLE 3

sequence (5'-3')	seqID	sequence (5'-3')	seqID	duplex name	single dose screen @ 25 nM (% residual mRNA)	SDs 2nd screen (among quadruplicates)
ccAuuAcuAcAGuAGcAccuTsT	582	AGUGCuACUGuAGuAAUGGTsT	583	AD-14085	19%	3%
AucuGGcAAccAuAuuucuTsT	584	ACAAAuAUGGUUGCcAGAUTsT	585	AD-14086	38%	1%
GAuAGCuAAAuuAAAccAATsT	586	UUGGUUuAAUUuAGCuAUCTsT	587	AD-14087	75%	10%
AGAuAccAuuAcuAcAGuATsT	588	uACUGuAGuAAUGGuAUCUTsT	589	AD-14088	23%	8%
GAuuGuucAucAAuuGGcGTsT	590	CGCcAAUUGAUGAAcAAUCTsT	591	AD-14089	70%	12%
GcuuucuccucGGcucAcuTsT	592	AGuGAGCCGAGGAGAAAGCTsT	593	AD-14090	79%	11%
GGAGCAuuGGcuGAcAAGATsT	594	UCUUGUcAGCcAAUCCUCCTsT	595	AD-14091	29%	3%
uAAuGAAGAGuAuAccuGGTsT	596	CcAGGuAuACUCUUcAUuATsT	597	AD-14092	23%	2%
uuucAccAAAccAuuuGuATsT	598	uAcAAAUGGUUUGGUGAAATsT	599	AD-14093	60%	2%
cuuAuuAAGGAGuAuAcGGTsT	600	CCGuAuACUCCUuAAuAAGTsT	601	AD-14094	11%	3%
GAAAucAGAuGCAcGuAAGTsT	602	CUuACGUCcAUCUGAUUUCTsT	603	AD-14095	10%	2%
cAGAuGucAGcAuAAGcCATsT	604	UCGCUuAUGCUGAcAUCUGTsT	605	AD-14096	37%	2%
AucuAAccccuAGuuGuAucTsT	606	GAuAcAACuAGGGUuAGAUTsT	607	AD-14097	45%	6%
AAGAGcuuGuuAAAAucGGTsT	608	CCGAUUUuAAcAAGCUCUUTsT	609	AD-14098	50%	10%
uuAAGCACuAuAcGCACGATsT	610	UCGUGCGuAuACUCCUuAATsT	611	AD-14099	12%	4%
uuGcAAuGuAAAuAcGuAuTsT	612	AuACGuAUUuAcAUUGcAATsT	613	AD-14106	49%	7%
ucuAAccccuAGuuGuAucuTsT	614	GGAuAcAACuAGGGUuAGATsT	615	AD-14101	36%	1%
cAuGuAucuuuuucucGAuTsT	616	AUCGAGAAAAAGAuAcAUGTsT	617	AD-14102	49%	3%
GAuGucAGcAuAAGcGAuGTsT	618	cAUCGCUuAUGCUGAcAUCTsT	619	AD-14103	74%	9%
ucccAACAGGuAcGAcAccTsT	620	GGUGUCGuACCUGUUGGGATsT	621	AD-14104	27%	3%
uGcucAcGAuGAGuuuAGuTsT	622	ACuAAACUcAUCGUGAGcATsT	623	AD-14105	34%	4%
AGAGcuuGuuAAAAucGGATsT	624	UCCGAUUUuAAcAAGCUCUTsT	625	AD-14106	9%	2%
GcGuAcAAGAAcAucuAuATsT	626	uAuAGAUGUUCUUUGuACGCTsT	627	AD-14107	8%	1%
GAGGuuGuAAGccAAuGuuTsT	628	AACAUUGGCUuAcAACCUCTsT	629	AD-14108	15%	1%
AAcAGGuAcGAcAccAcAGTsT	630	CUGUGGUGUCGuACCUGUUTsT	631	AD-14109	91%	2%
AAcccuAGuuGuAucccucTsT	632	GAGGGAuAcAACuAGGGUUTsT	633	AD-14110	66%	5%
GcAuAAGcGAuGGAuAAuATsT	634	uAUuAUCcAUCGCUuAUGCTsT	635	AD-14111	33%	3%
AAGcGAuGGAuAAuAccuATsT	636	uAGGuAUuAUCcAUCGCUUTsT	637	AD-14112	51%	3%
uGAucccuGuAcGAAAAGAATsT	638	UUCUUUUCGuAcAGGAUcATsT	639	AD-14113	22%	3%
AAAAcAuuGGccGuucuGGTsT	640	CcAGAACGGCcAAUGUUUUTsT	641	AD-14114	117%	8%
cuuGGAGGGcGuAcAACAATsT	642	UUGUUGuACGCCCUCcAAGTsT	643	AD-14115	50%	8%
GGcGuAcAAGAAcAucuAuTsT	644	AuAGAUGUUCUUGuACGCCTsT	645	AD-14116	14%	3%
AcucuGAGuAcAuuGGAuTsT	646	AUCcAAUGuACUcAGAGUTsT	647	AD-14117	12%	4%
uuAuuAAGCAGuAuAcGGATsT	648	UCCGuAuACUCCUuAAuAATsT	649	AD-14118	26%	4%
uAAGCAGuAuAcGGAGGAGTsT	650	CUCCUCCGuAuACUCCUuATsT	651	AD-14119	24%	5%
AAAucAAuAGucAAcuAAATsT	652	UUuAGUUGACuAUUGAUUUTsT	653	AD-14120	8%	1%
AAucAAuAGucAAcuAAAGTsT	654	CUUuAGUUGACuAUUGAUUTsT	655	AD-14121	24%	2%
uucucAGuAuAcuGuGuAATsT	656	UuAcAcAGuAuACUGAGAATsT	657	AD-14122	10%	1%
uGuGAAAcAcucuGAuAAATsT	658	UUuAUcAGAGUGUUUcAcATsT	659	AD-14123	8%	1%
AGAuGuGAAucucuGAAcATsT	660	UGUUcAGAGAUUcAcAUCUTsT	661	AD-14124	9%	2%
AGGuuGuAAGccAAuGuuGTsT	662	cAACAUUGGCUuAcAACCUTsT	663	AD-14125	114%	5%
uGAGAAAucAGAuGCAcGuTsT	664	ACGUCcAUCUGAUUUCUcATsT	665	AD-14126	9%	1%
AGAAAucAGAuGCAcGuAATsT	666	UuACGUCcAUCUGAUUUCUTsT	667	AD-14127	57%	6%
AuAucccAAcAGGuAcGAcTsT	668	GUCGuACCUGUUGGGAuAUTsT	669	AD-14128	104%	6%
cccAAcAGGuAcGAcAccATsT	670	UGGUGUCGuACCUGUUGGGTsT	671	AD-14129	21%	2%
AGcAuAcuGAAGAAccucuTsT	672	AGAGGUUCUUcAGuAuGCUTsT	673	AD-14130	57%	6%
AuAuAuAucAGccGGGcGcTsT	674	GCGCCCGGCUGAuAuAuAUTsT	675	AD-14131	93%	6%
AAucuAAcccuAGuuGuAuTsT	676	AuAcAACuAGGGUuAGAUUTsT	677	AD-14132	75%	8%
cuAAcccuAGuuGuAucccTsT	678	GGGAuAcAACuAGGGUuAGTsT	679	AD-14133	66%	4%
cuAGuuGuAucccucccuuTsT	680	AAAGGAGGGAuAcAACuAGTsT	681	AD-14134	44%	6%
AGAcAucuCAcuAAuGGcuTsT	682	AGCcAUuAGUcAGAUGUCUTsT	683	AD-14135	55%	6%

TABLE 3

GAAGcucAcAAuGAcuuAATsT	684	UuAAAUcAUUGUGAGCUUCTsT	685	AD-14136	29%	3%
AcAuGuAucuuuuucucGATsT	686	UCGAGAAAAAGAuAcAUGUTsT	687	AD-14137	40%	3%
ucGAuucAAAucuuAAccCTsT	688	GGGUuAAGAUUUGAAUCGATsT	689	AD-14138	38%	5%
ucuuAAccuuAGGAcucuTsT	690	AGAGUCCuAAGGGUuAAGATsT	691	AD-14139	71%	11%
GcucAcGAuGAGuuuAGuGTsT	692	cACuAAACUcAUCGUGAGCTsT	693	AD-14140	43%	15%
cGuAAGcGuuGGAuAAuAcTsT	694	GuAUuAUCcAUCGCUuAUGTsT	695	AD-14141	33%	6%
AuGAGcGAuGGAuAAuAccTsT	696	GGuAUuAUCcAUCGCUuAUTsT	697	AD-14142	51%	14%
ccuAAuAAAcuGccucucAGTsT	698	CUGAGGGcAGUUuAUuAGGTsT	699	AD-14143	42%	1%
ucGGAAGuuGAAcuuGCuTsT	700	ACGAAGUUcAACUUUCCGATsT	701	AD-14144	4%	4%
GAAAAcAuuCGccGuucuGTsT	702	cAGAACGCCcAAUGUUUUCTsT	703	AD-14145	92%	5%
AAGAcuGAucuucuAAGuuTsT	704	AACUuAGAAGAUcAGUCUUTsT	705	AD-14146	13%	2%
GAGcuuGuuAAAAucGCAATsT	706	UUCCGAUUUuAAcAAGCUCTsT	707	AD-14147	8%	1%
AcAuuGGccGuucuGGAGcTsT	708	GCUCcAGAACGGCcAAUCUTsT	709	AD-14148	80%	7%
AAGAAcAuucuAuAAuuGcATsT	710	UGcAAUuAuAGAUGUUCUUTsT	711	AD-14149	44%	7%
AAAuGuGucuAcucAuGuuTsT	712	AAcAUGAGuAGAcAcAUUUTsT	713	AD-14150	32%	29%
uGucuAcucAuGuuucucATsT	714	UGAGAAAcAUGAGuAGAcATsT	715	AD-14151	75%	11%
GuAuAcuGuGuAcAAucuTsT	716	AGAUUGUuAcAcAGuAuACTsT	717	AD-14152	8%	5%
GAuAcuGuGuAcAAucuATsT	718	uAGAUUGUuAcAcAGuAuATsT	719	AD-14153	17%	11%
cuuAGuAGuGucuAGGAAATsT	720	UUUCCUGGAcACuACuAAGTsT	721	AD-14154	16%	4%
ucAGAuGGAcGuAAGGcAGTsT	722	CUGCCUuACGUCcAUCUGATsT	723	AD-14155	11%	1%
AGAuAAAuuGAuAGcAcAATsT	724	UUGUGCuAUcAAUUuAGCUTsT	725	AD-14156	10%	1%
cAAcAGGuAcGAcAccAcATsT	726	UGUGGUGUCGuACCUGUUGTsT	727	AD-14157	39%	3%
uGcAAuGuAAAuAcGuAuuTsT	728	AAuACGuAUUuAcAUUGcATsT	729	AD-14158	51%	3%
AGucAGAAuuuuAuucuAGATsT	730	UCuAGAuAAAAUUCUGACUTsT	731	AD-14159	93%	5%
cuAGAAAucuuuuAAcAccTsT	732	GGUGUuAAAAGAUUUCuAGTsT	733	AD-14160	40%	3%
AAuAAAucuAAcccuAGuuTsT	734	AACuAGGGUuAGAUUuAUUTsT	735	AD-14161	83%	7%
AAuuuucuGGucAcGAuGATsT	736	UcAUCGUGAGcAGAAAAUUTsT	737	AD-14162	44%	6%
GcccucAGuAAAuccAuGGTsT	738	CcAUGGAUUuACUGAGGGCTsT	739	AD-14163	57%	3%
AcGuuuAAAAcGAGAucuuTsT	740	AAGAUCUCGUUUuAAACGUTsT	741	AD-14164	4%	1%
ACGAGAuAGAAcGuuuAAATsT	742	UUuAAACGUUCuAUCUCCUTsT	743	AD-14165	11%	1%
GAccGucAuGcGucGcGcAGTsT	744	CUGCGACGCcAUGACGGUCTsT	745	AD-14166	90%	5%
AccGucAuGcGucGcGcAGCTsT	746	GCUGCGACGCcAUGACGGUTsT	747	AD-14167	49%	1%
GAAcGuuuAAAcGAGAucTsT	748	GAUCUCGUUUuAAACGUUCTsT	749	AD-14168	12%	2%
uuGAGcuuAAcAuAGGuAATsT	750	UuACCuAUGUuAAGCUcAATsT	751	AD-14169	66%	4%
ACuAAAuuGuucucGuAGATsT	752	UCuACGAGAUcAAUUuAGUTsT	753	AD-14170	52%	6%
ucGuAGAuuAucuuAAuATsT	754	uAUuAAGAuAAUUCuACGATsT	755	AD-14171	42%	4%
GUuGAuAGAAcGuuuAAATsT	756	UUUuAAACGUUCuAUCUCCTsT	757	AD-14172	3%	1%
AcAAcuuAuuGGAGGuuGuTsT	758	AcAACCUCcAAuAAGUUGUTsT	759	AD-14173	39%	2%
uGAGcuuAAcAuAGGuAAATsT	760	UUuACCuAUGUuAAGCUcATsT	761	AD-14174	69%	2%
AucucGuAGAAuuAucuuATsT	762	uAAGAuAAUUCuACGAGAUTsT	763	AD-14175	53%	3%
cuGcGuGcAGucGGucGucTsT	764	GACGACCGACUGcACGcAGTsT	765	AD-14176	111%	4%
cAcGcAGcGccGAGAGuATsT	766	uACUCUCGGGCGCUGCGUGTsT	767	AD-14177	87%	6%
ACuAccAGGGAGAcucccGTsT	768	CGGGAGUCUCCCUGGuACUTsT	769	AD-14178	59%	2%
AcGGAGGAGAuAGAcGuuTsT	770	AACGUUCuAUCUCCUCCGUTsT	771	AD-14179	9%	2%
AGAAcGuuuAAAAcGAGAuTsT	772	AUCUCGUUUuAAACGUUCUTsT	773	AD-14180	43%	2%
AAcGuuuAuAAcGAGAucuTsT	774	AGAGCUCGUUUuAAACGUUTsT	775	AD-14181	70%	10%
AGcuuGAGcuuAAcAuAGTsT	776	CCuAUGUuAAGCUcAAGCUTsT	777	AD-14182	100%	7%
AGcuuAAcAuAGGuAAAuATsT	778	uAUUuACCuAUGUuAAGCUTsT	779	AD-14183	60%	5%
uAGAGcuuAcAAAAcGuAucTsT	780	GAuACGUUUUGuAAGCUCuATsT	781	AD-14184	128%	6%
uAGuuGuAucccuccuuuATsT	782	uAAAGGAGGGAuAcAACuATsT	783	AD-14185	62%	4%
AccAcccAGAcAucuGAcuTsT	784	AGUcAGAUGUCUCGGGUGGUTsT	785	AD-14186	42%	3%
AGAAAcuAAAuuGAucucTsT	786	CGAGAUcAAUUUuAGUUUCUTsT	787	AD-14187	123%	12%
ucucGuAGAAuuAucuuAATsT	788	UuAAGAuAAUUCuACGAGATsT	789	AD-14188	38%	3%
cAAcuuAuuGGAGGuuGuATsT	790	uAcAACCUCcAAuAAGUUGUTsT	791	AD-14189	13%	1%
uuGuAucccuccuuuAAGuTsT	792	ACUuAAAGGAGGGAuAcAATsT	793	AD-14190	59%	3%
ucAcAAcuuAuuGGAGGuuTsT	794	AACCUCcAAuAAGUUGUGATsT	795	AD-14191	93%	3%

TABLE 3

AGAAcuGuAcucuucucAGTsT	796	CUGAGAAGAGuAcAGUUCUTsT	797	AD-14192	45%	5%
GAGcuuAAcAuAGGuAAAuTsT	798	AUUuACCuAUGUuAAGCUCTsT	799	AD-14193	57%	3%
cAccAAcAucuGucomuAGTsT	800	CuAAGGAcAGAUGUUGGUGTsT	801	AD-14194	51%	4%
AAAGCcCACuuuAGAGuAuTsT	802	AuACUCuAAAGUGGGCUUUTsT	803	AD-14195	77%	5%
AAGcccAcuuuAGAGuAuATsT	804	uAuACUCuAAAGUGGGCUUTsT	805	AD-14196	42%	6%
GAccuuAuuuGGuAAucuGTsT	806	cAGAUuACcAAAuAAGGUCTsT	807	AD-14197	15%	2%
GAuuAAuGcAcucAAGAcuTsT	808	AGUCUUGAGuAcAUuAAUCTsT	809	AD-14198	12%	2%
cuuuAAGAGCccuAAcucATsT	810	UGAGUuAGGCCUCUuAAAGTsT	811	AD-14199	18%	2%
uuAAAccAAAccuAuuGATsT	812	UcAAuAGGGUUUGGUUuAATsT	813	AD-14200	73%	9%
ucuGuuGGAGAucuAuAAuTsT	814	AUuAuAGAUCUCcAAcAGATsT	815	AD-14201	9%	3%
cuGAuGuuucuGAGAGAcuTsT	816	AGUCUCUcAGAAAcAUcAGTsT	817	AD-14202	25%	3%
GcAuAcucuAGucGuuzccTsT	818	GGGAACGACuAGAGuAUGCTsT	819	AD-14203	31%	1%
GuuccuuAucGAGAAucuATsT	820	uAGAUUCUCGAuAAGGAACTsT	821	AD-14204	8%	2%
GcAcuuGGAucucucAcAuTsT	822	AUGUGAGAGAUCcAAGUGCTsT	823	AD-14205	5%	1%
AAAAAAGAAAcuAGAuGCcTsT	824	GCcAUCuAGUUCCUUUUUUTsT	825	AD-14206	79%	6%
AGAGcAGAuuAccucucuGcGTsT	826	CGcAGAGAGGuAAUCUGCUCTsT	827	AD-14207	55%	2%
AGcAGAuuAccucucuGcGAGTsT	828	CUCGcAGAGGuAAUCUGCUTsT	829	AD-14208	100%	4%
ccccuGAcAGAGuucAcAAATsT	830	UUUGUGAACUCUGUcAGGGTsT	831	AD-14209	34%	3%
GuuuAccGAAGuGuuGuuuTsT	832	AAACAACACUUCGGuAAACTsT	833	AD-14210	13%	2%
uuAcAGuAcAcAAcAAGAATsT	834	UCCUUGUUGUGuACUGuAATsT	835	AD-14211	9%	3%
AcuGGAucGuAAGAAGGcATsT	836	UGCCUUCUuACGAUCcACUTsT	837	AD-14212	30%	3%
GAGcAGAuuAccucucGcGATsT	838	UCGcAGAGGuAAUCUGCUCTsT	839	AD-14213	46%	5%
AAAAGAAGuuAGuGuAcGATsT	840	UCGuAcACuAACUUCUUUUTsT	841	AD-14214	38%	18%
GAccAuuuAAuuuGGcAGATsT	842	UCUGCcAAAUuAAAUGGUCTsT	843	AD-14215	132%	6%
GAGAGGAGuCAuAAuuAAATsT	844	UUuAAUuAUcACUCCUCUCTsT	845	AD-14216	3%	0%
cuGGAGGAuuGGcuGAcAATsT	846	UUGUcAGCcAAUCCUCcAGTsT	847	AD-14217	19%	1%
cucuAGucGuucccAcucATsT	848	UGAGUGGGAACGACuAGAGTsT	849	AD-14218	67%	8%
GAuAccAuuAcuAcAGuAGTsT	850	CuACUGuAGuAAUGGuAUCTsT	851	AD-14219	76%	4%
uucGucuGccAAGAACAAATsT	852	UUUCUUCUUCGcAGACGAATsT	853	AD-14220	33%	8%
GAAAAGAAGuuAGuGuAcGTsT	854	CGuAcACuAACUUCUUUUCTsT	855	AD-14221	35%	2%
uGAuGuuuAccGAAGuGuuTsT	856	AAcACUUCGGuAAAcAUcATsT	857	AD-14222	7%	2%
uGuuAcucAAuucuGGAuTsT	858	AUCcAGAAUUGUAcAAAcATsT	859	AD-14223	19%	2%
AuGAAGAGuAuAccuUGGATsT	860	UCCcAGGuAuACUCUuCAUTsT	861	AD-14224	13%	1%
GcuAcucuGAuGAAuGcAuTsT	862	AUGcAUUcAUcAGAGuAGCTsT	863	AD-14225	15%	3%
GCGGuuGuAGAAAGAAcAcTsT	864	GUGUUCUUUCuAcAAGGGCTsT	865	AD-14226	12%	0%
ucAuGuucGuuAucGAGAATsT	866	UUCUCGAuAACGAAcAUGATsT	867	AD-14227	5%	1%
GAAuAGCGuuAcAGAGuuGTsT	868	cAACUCUGuAACGCuAUUCTsT	869	AD-14228	34%	3%
cAAAcuGCAucGuAAGAAGTsT	870	CUUCUuACGAUCcAGUUUGTsT	871	AD-14229	15%	2%
cuuAuuuGCuAAucuGcuGTsT	872	cAGcAGAUuACcAAAuAAGTsT	873	AD-14230	20%	1%
AGcAAuGuGUAAAcuAAcTsT	874	GUuAGGUUUCcAcAUUGCUTsT	875	AD-14231	19%	1%
AcAAuAAAGcAGAcccAuuTsT	876	AAUGGGUCUGCUUuAUUUGUTsT	877	AD-14232	21%	1%
AAccAcuuAGuAGuGucCATsT	878	UGGAcACuACuAAGUGGUUTsT	879	AD-14233	106%	12%
AGucAAGAGccAucuGuAGTsT	880	CuAcAGAUGGCUCUUGACUTsT	881	AD-14234	35%	3%
cuccccuAGAcuuccccuAuuTsT	882	AAuAGGGAAGUCuAGGGAGTsT	883	AD-14235	68%	4%
AuAGcuAAAuuAAAccAAATsT	884	UUUGGUUuAAUUuAGCuAUTsT	885	AD-14236	22%	3%
uGGcuGGuAuAAuuccAcGTsT	886	CGUGGAAUuAuACcAGCcATsT	887	AD-14237	75%	9%
uuAuuuGCuAAucuGcuGuTsT	888	AcAGcAGAUuACcAAAuAATsT	889	AD-14238	92%	7%
AAcuAGAuGGcuuuucucAGTsT	890	CUGAGAAAGCcAUCuAGUUTsT	891	AD-14239	20%	2%
ucAuGGcGuccGcAGccAAATsT	892	UUUGGCUGCGACGCcAUGATsT	893	AD-14240	71%	6%
AcuGCAGGAuuGGcuGAcATsT	894	UGUcAGCcAAUCCUCcAGUTsT	895	AD-14241	34%	3%
cuAuAuuGcAcuAucuuuTsT	896	AAAGAuAGUGcAAUuAuAGTsT	897	AD-14242	11%	2%
AAGGucAccuAuuGAAGATsT	898	UCUUcAUuUGGUGACCUUUTsT	899	AD-14243	11%	1%
AuGAuGcAuAcucuAGucTsT	900	GACuACAGuAUGcAUUcAUTsT	901	AD-14244	15%	2%
AAcAuAuuGAAuAAGccuGTsT	902	cAGGCUuAUUcAUuAUGUUTsT	903	AD-14245	80%	7%
AGGAAGGcAGuuGAccAAcTsT	904	GUUGGUcAACUGCCUUCUUTsT	905	AD-14246	57%	5%
GAuAcuAAAAGAAcAAuAATsT	906	UGAUUGUUCUUUuAGuAUCTsT	907	AD-14247	9%	3%

TABLE 3

AuAcuGAAAAucAAuAGucTsT	908	GACuAUUGAUUUUcAGuAUTsT	909	AD-14248	39%	4%
AAAAAGGAAccuAGAuGGcuCTsT	910	AGCcAUCuAGUUCCUUUUUTsT	911	AD-14249	64%	2%
GAAcuAGAuGGcuuucucATsT	912	UGAGAAAGCcAUCuAGUUCTsT	913	AD-14250	18%	2%
GAAAccuAAcuGAAGAccuTsT	914	AGGUCUUCAGUuAGGUUUCTsT	915	AD-14251	56%	6%
uAccAucAAGAcuGGuAATsT	916	UuACcAGUGUUGAUGGGuATsT	917	AD-14252	48%	6%
AuuuuGAuAucuAccuAuuTsT	918	AAUGGGuAGAuAUcAAAAUTsT	919	AD-14253	39%	5%
AucccuAuAGuucAcuuuGTsT	920	cAAAGUGAACuAuAGGGAUTsT	921	AD-14254	44%	8%
AuGGGcuAuAAcuGcAcuATsT	922	uAGUGcAAUuAuAGCCcAUTsT	923	AD-14255	108%	8%
AGAuuAccucuGcGAGccTsT	924	GGCUCGcAGAGGuAAUCUTsT	925	AD-14256	108%	6%
uAAuucAcGuAccuucATsT	926	UGAAGGuACGUGGAAUuATsT	927	AD-14257	23%	2%
GucGuucGcAcucAGuuuuTsT	928	AAAACuGAGuGCGAACGACTsT	929	AD-14258	21%	3%
AAAucAAucccuGuuGAcuTsT	930	AGUcAAcAGGGAUUGAUUUTsT	931	AD-14259	19%	2%
ucAuAGAGcAAGAACAuATsT	932	uAUGUUCUUUGCUCuAUGATsT	933	AD-14260	10%	1%
uuAcuAcAGuAGcAcuuGGTsT	934	CcAAGUGCuACUGuAGuAATsT	935	AD-14261	76%	3%
AuGuGGAAAccuAAcuGAATsT	936	UUcAGUuAGGUUUCcAcAUTsT	937	AD-14262	13%	2%
uGuGGAAAccuAAcuGAAGTsT	938	CUUcAGUuAGGUUUCcAcATsT	939	AD-14263	14%	2%
ucuucucuuAAAuGAAAGGTsT	940	CCCUUUcAUUuAAGGAAGATsT	941	AD-14264	65%	3%
uGAAGAAcucuucAACucAATsT	942	UUGACUuAGAGGUUCUUcATsT	943	AD-14265	13%	1%
AGAGCucuAAAGuGAAGATsT	944	UCUUCcACUUuAGACCUCUTsT	945	AD-14266	18%	3%
AuAucuAccuAuuuucuGTsT	946	cAGAAAAAUGGCuAGAuAUTsT	947	AD-14267	50%	9%
uAAGccuGAAGuGAAucAGTsT	948	CUGAUUcACUUcAGGCUuATsT	949	AD-14268	13%	3%
AGAuGcAGAccAuuuAAuuTsT	950	AAUuAAAUGGUCUGcAUCUTsT	951	AD-14269	19%	4%
AGuGuuGuuuGuccAAuucTsT	952	GAAUUGGAcAAAcAAcACUTsT	953	AD-14270	11%	2%
cuAuAAuGAAGAGcuuuuuTsT	954	AAAAAGCUCUUcAUuAuAGTsT	955	AD-14271	11%	1%
AGAGGAGuGAuAAuuAAAGTsT	956	CUUuAAUuAUcACUCCUCUTsT	957	AD-14272	7%	1%
uuucucuGuuAcAAuAcAuGTsT	958	AUGuAUUCuAAcAGAGAAATsT	959	AD-14273	14%	2%
AAcAucuAuAAAuuGcAAcATsT	960	UGUUGcAAUuAuAGAUGUUTsT	961	AD-14274	73%	4%
uGccuAGAAGuAcAuAAGAcTsT	962	GUCUuAUGuACUUCuAGcATsT	963	AD-14275	10%	1%
AAuGuAcucAAGAcuGAucTsT	964	GAUcAGUCUUGAGuAcAUUTsT	965	AD-14276	89%	2%
GuAcucAAGAcuGAucuucTsT	966	GAAGAUcAGUCUUGAGuACTsT	967	AD-14277	7%	3%
cAcucuGAuAAAcucAAuGTsT	968	cAUUGAGUUuAUcAGAGUGTsT	969	AD-14278	12%	3%
AAGAGcAGAuuAccucuGcTsT	970	GcAGAGGuAAUCUGCUCUUTsT	971	AD-14279	104%	3%
ucuGcGAGccuGGAucAAcTsT	972	GUUGAUCUGGGCUCCGcAGATsT	973	AD-14280	21%	2%
AAcuuGAGccuuGuGuGuATsT	974	uAuACACAAGGCCUcAAGUUTsT	975	AD-14281	43%	3%
GAAuAuAuAuAucAGccGGTsT	976	CCGGCUGAuAuAuAuAUUCTsT	977	AD-14282	45%	6%
uGucAucccuAuAGuucAcTsT	978	GUGAACuAuAGGGAUGAcATsT	979	AD-14283	35%	5%
GAucuGGcAAccAuAuuucTsT	980	GAAAuAUGGUUGCcAGAUCTsT	981	AD-14284	58%	3%
uCGcAAccAuAuuucuGGATsT	982	UCcAGAAAuAUGGUUGCcATsT	983	AD-14285	48%	3%
GAuGuuuAccGAAGuGuuGTsT	984	cAACACUUCGuAAACAUCTsT	985	AD-14286	49%	3%
uuccuuAuuGAGAAucuAATsT	986	UuAGAUUCUCGAuAAGGAATsT	987	AD-14287	6%	1%
AGccuAAuuGcuuucuGGATsT	988	UCcAGAAAGcAAUuAAGCUTsT	989	AD-14288	50%	2%
uuGcuAuuAuGGGAGAccATsT	990	UGGUCUCCcAuAAuAGcAATsT	991	AD-14289	48%	1%
GucAuGCcGucGcAGccAATsT	992	UUGGCUGCGACGCcAUGACTsT	993	AD-14290	112%	7%
uAAuuGcAcuAuuuuuGcGTsT	994	CGcAAAGAuAGUGcAAUuATsT	995	AD-14291	77%	2%
cuAucuuuGcGuAuGcGcCATsT	996	UGGCcAuACGcAAAGAuAGTsT	997	AD-14292	80%	6%
ucccuAuAGuucAcuuuGuTsT	998	AcAAAGUGAACuAuAGGGATsT	999	AD-14293	58%	2%
uuAAccuuuAAuucAcuuGTsT	1000	cAAGUGAAUuAAAGUUGATsT	1001	AD-14294	77%	2%
GGcAAccAuAuuucuGGAATsT	1002	UUCcAGAAAuAUGGUUGCCTsT	1003	AD-14295	62%	2%
AuGuAcucAAGAcuGAucuTsT	1004	AGAUcAGUCUUGAGuACAUTsT	1005	AD-14296	59%	4%
GcAGAccAuuuAAuuuGGcTsT	1006	GCcAAAUuAAAUUGGUCUGCTsT	1007	AD-14297	37%	1%
ucuGAGAGAcuAcAGAuGuTsT	1008	AcAUCUGuAGUCUCUcAGATsT	1009	AD-14298	21%	1%
uCcucAuAGAGcAAAGAAcTsT	1010	GUUCUUUCCUCuAUGAGcATsT	1011	AD-14299	6%	1%
AcAuAAGAccuuAuuuGGuTsT	1012	ACcAAAuAAGGUCUuAUGUTsT	1013	AD-14300	17%	2%
uuuGuGcuGAuucuGAuGGTsT	1014	CcAUcAGAAUcAGcAcAAATsT	1015	AD-14301	97%	6%
ccAucAAcAcuGGuAAGAATsT	1016	UUCUuACcAGUGUUGAUGGTsT	1017	AD-14302	13%	1%
AGAcAAuucGGAuGuGGATsT	1018	UCcAcAUCCCGAAUUGUCUTsT	1019	AD-14303	13%	3%

TABLE 3

GAAcuuGAGccuuGuGuAuTsT	1020	AuAcAcAAGGCUcAAGUUCTsT	1021	AD-14304	38%	2%
uAAuuuGCcAGAGcGGAAATsT	1022	UUUCCGCUCUCUGCcAAAUuATsT	1023	AD-14305	14%	2%
uGGAuGAAGuuAuuAuGGTsT	1024	CCcAuAAuAACUUcAUCcATsT	1025	AD-14306	25%	4%
AuCuAcAuGAAcuAcAAGATsT	1026	UCUUGuAGUUcAUGuAGAUTsT	1027	AD-14307	26%	6%
GCuAuuuuuGAucuGGcAATsT	1028	UUGCcAGAUcAAAAAuACCTsT	1029	AD-14308	62%	8%
cuAAuGAAGAGuAuAccuGTsT	1030	cACGuAuACUCUUcAUuAGTsT	1031	AD-14309	52%	5%
uuuGACAAAcuuAcuGAuATsT	1032	uAUcAGuAAGUUUCUcAAATsT	1033	AD-14310	32%	3%
cGAuAAGAuAGAAGAucAATsT	1034	UUGAUCUUCuAUCUuAUCGTsT	1035	AD-14311	23%	2%
cuGGcAAccAuAuuucuGGTsT	1036	CcAGAAAuAUGGUUUCcAGTsT	1037	AD-14312	49%	6%
uACAuAccAuuAcuAcAGuTsT	1038	ACUGuAGuAAUCGuAUCuATsT	1039	AD-14313	69%	4%
GuAuAAAuuGGGuuucAuTsT	1040	AUGAAACCcAAUUuAAuACTsT	1041	AD-14314	52%	3%
AAGAccuuAuuuGCuAAucTsT	1042	GAUuACcAAAuAAGGUCUUTsT	1043	AD-14315	66%	4%
GcuGuuGAuAAGAGAGcucTsT	1044	GAGCUCUCUuAUcAAcAGCTsT	1045	AD-14316	19%	4%
uAcucAuGuuucucAGAuuTsT	1046	AAUCUGAGAAACAUGAGuATsT	1047	AD-14317	16%	9%
cAGAuGGAcAuAAGGcAGcTsT	1048	GCUGCCUuACGUCcAUCUGTsT	1049	AD-14318	52%	11%
uAucccAAcAGGuAcGAcATsT	1050	UGUCGuACCUGUUUGGGAuATsT	1051	AD-14319	26%	11%
cAuuGcuAuuAuGGGAGAcTsT	1052	GUCUCCcAuAAuAGcAAUGTsT	1053	AD-14320	52%	10%
ccccucAGuAAAuccAuGGuTsT	1054	ACcAUGGAUUuACUGAGGGTsT	1055	AD-14321	53%	6%
GCuGAuuAcuGCCcuuGuATsT	1056	uAcAAGGGcAGuAADGACCTsT	1057	AD-14322	30%	2%
AAccAcucAAAAcAuuuGTsT	1058	cAAAUGUUUUUGAGUGGUUTsT	1059	AD-14323	116%	6%
uuuGcAAGucAAuGAAucuTsT	1060	AGAUUcAUuAACUUGcAAATsT	1061	AD-14324	14%	3%
uuAuuuucAGuAGucAGAATsT	1062	UUCUGACuACUGAAAAuAATsT	1063	AD-14325	90%	2%
uuuucucGuucAAAucuuTsT	1064	AAGAUUuGAAUCGAGAAAATsT	1065	AD-14326	47%	3%
GuAcGAAAAGAAGuuAGuGTsT	1066	cACuAACUUCUUUUCGuACTsT	1067	AD-14327	18%	2%
uuuAAAAcGAGAucuuGcuTsT	1068	AGcAAGAUCUCGUUUuAAATsT	1069	AD-14328	19%	1%
GAAuuGAuuAAuGuAcucATsT	1070	UGAGuAcAUuAAUcAAUUCTsT	1071	AD-14329	94%	10%
GAuGGAcGuAAGGcAGcucTsT	1072	GAGCUGCCUuACGUCcAUCTsT	1073	AD-14330	60%	4%
cAucuCAcuAAuGGcucuGTsT	1074	cAGAGCcAUuA3UcAGAUGTsT	1075	AD-14331	54%	7%
GuGAucccuGuAcGAAAGATsT	1076	UCUUUCGuAcA3GAUcACTsT	1077	AD-14332	22%	4%
AGcucuuAuuAAGGAGuAuTsT	1078	AuACUCCUuAAuAAGAGCUTsT	1079	AD-14333	70%	10%
GcucuuAauAAGGAGuAuATsT	1080	uAuACUCCUuAAuAAGAGCTsT	1081	AD-14334	16%	3%
ucuuAuAAAGGAGuAuAcGTsT	1082	CGuAuACUCCUuAAuAAGATsT	1083	AD-14335	38%	6%
uAuuAAGGAGuAuAcGGAGTsT	1084	CUCCGuAuACUCCUuAAuATsT	1085	AD-14336	16%	3%
cuGcAGccuGuGAGAAAAATsT	1086	UUUUUCUcACGGGCUGcAGTsT	1087	AD-14337	65%	4%
ucAAGAcuGAucuuucuAAGTsT	1088	CUuAGAAGAUcAGUCUUGATsT	1089	AD-14338	18%	0%
cuucuAAGuucAcuGGAAATsT	1090	UUUCcAGUGAACUuAGAAGTsT	1091	AD-14339	20%	4%
uGcAAGuuAAuGAAUCuuuTsT	1092	AAAGAUUcAUuAACUUGcATsT	1093	AD-14340	24%	1%
AAucuAAGAuAuAGucAATsT	1094	UUGACuAuAUCCUuAGAUUTsT	1095	AD-14341	37%	3%
AucucuGAAcAcAAGAAcATsT	1096	UGUUCUUGUGUUCAGAGAUTsT	1097	AD-14342	13%	1%
uucuCAAcAGuGGuAucuTsT	1098	AGAuACCcACUGUUcAGAATsT	1099	AD-14343	19%	1%
AGuuAuuAuAcccAucAATsT	1100	UUGAUGGGuAuAAAuAACUTsT	1101	AD-14344	23%	2%
AuGcuAAAcuGuucAGAAATsT	1102	UUUCUGAAcAGUUuAGcAUTsT	1103	AD-14345	31%	4%
cuAcAGAGcAcuuGAuAcTsT	1104	GuAAGcAAGUGCUCUGuAGTsT	1105	AD-14346	18%	2%
uAuAuAucAGccGGcGcGTsT	1106	CGCGCCCGGCUGAuAuAuATsT	1107	AD-14347	67%	2%
AuGuAAAuACGuAuuucuATsT	1108	uAGAAAuACGuAUUuAcAUTsT	1109	AD-14348	39%	3%
uuuuucucGAuucAAAucuTsT	1110	AGAUUuGAAUCGAGAAAATsT	1111	AD-14349	83%	6%
AAucuuAAccuuuAGGAcuTsT	1112	AGUCCuAAGGGUuAAGAUUTsT	1113	AD-14350	54%	2%
ccuuAGGAcuucuGGuAuuuTsT	1114	AAAuACcAGAGUCCuAAGGTsT	1115	AD-14351	57%	8%
AAuAAAcuGcccucAGuAATsT	1116	UuACUGAGGGcAGUUuAUUTsT	1117	AD-14352	82%	3%
GAucccuGuAcGAAAGAAATsT	1118	CUUUCUUUCGuAcAGGAUCTsT	1119	AD-14353	2%	1%
AAuGuGAucccuGuAcGAAATsT	1120	UUUCGuAcAGGAUcAcAUUTsT	1121	AD-14354	18%	11%
GuGAAAcAuuGGccGuucTsT	1122	GAACGGCcAAUGUUUUcACTsT	1123	AD-14355	2%	3%
cuuGAGGAAAcucuGAGuATsT	1124	uACUcAGAGUUUCCUcAAGTsT	1125	AD-14356	8%	2%
cGuuuAAAAcGAGAucuuGcuTsT	1126	cAAGAUCUCGUUUuAAACGTsT	1127	AD-14357	6%	3%
uuAAAAcGAGAucuuGcuCTsT	1128	cAGcAAGAUCUCGUUUuAATsT	1129	AD-14358	98%	17%
AAAGAuGuAucuGGucucuTsT	1130	GGAGACcAGAuACAUCUUUTsT	1131	AD-14359	10%	3%

TABLE 3

cAGAAAAuGuGucuAcucATsT	1132	UGAGuAGAcAcAUUUUCUGTsT	1133	AD-14360	6%	4%
cAGAAAuGuGuuAAuGuAcTsT	1134	GuAcAUuAAUGcAAUCCUGTsT	1135	AD-14361	30%	5%
AGucAAcuAAAGcAuAuuuTsT	1136	AAAuAUGCUUuAGUUGACUTsT	1137	AD-14362	28%	2%
uGuGuAAcAAucuAcAuGATsT	1138	UcAUGuAGAUUGUuAcAcATsT	1139	AD-14363	60%	6%
AuAccAuuuGuucccuuGGuTsT	1140	ACcAAGGAAcAAAUGGuAUTsT	1141	AD-14364	12%	9%
GcAGAAAucuAAGGAuAuATsT	1142	uAuAUCCUuAGAUUUCUGCTsT	1143	AD-14365	5%	2%
uGGcuucucAcAGGAAcucTsT	1144	GAGUUCCUGUGAGAAGCcATsT	1145	AD-14366	28%	5%
GAGAuGuGAAucucuGAAcTsT	1146	GUUcAGAGAUUcAcAUCUCTsT	1147	AD-14367	42%	4%
uGuAAGccAAuGuuGuGAGTsT	1148	CUcAcAAcAUUGGCUuAcATsT	1149	AD-14368	93%	12%
AGccAAuGuuGuGAGGcuuTsT	1150	AAGCCUcAcAAcAUUGGCUTsT	1151	AD-14369	69%	4%
uUGUGAGGcuucAAGuucATsT	1152	UGAACUUGAAGCCUcAcAATsT	1153	AD-14370	5%	2%
AGGcAGcucAuGAGAAAcATsT	1154	UGUUUCUcAUGAGCUGCCUTsT	1155	AD-14371	54%	5%
AuAAAuuGAuAGcAcAAAATsT	1156	UUUUGUGCuAUcAAUUuAUTsT	1157	AD-14372	4%	1%
AcAAAAucuAGAAcuuAAuTsT	1158	AUuAAGUUCuAGAUUUUGUTsT	1159	AD-14373	5%	1%
GAuAuccccAAcAGGuAcGATsT	1160	UCGuACCUGUUUGGGAuAUCTsT	1161	AD-14374	92%	6%
AAGuuAuuuAuAcccAuuATsT	1162	UGAUGGGuAuAAAuAACUUTsT	1163	AD-14375	76%	4%
uGuAAAuAcGuAuuucuuGTsT	1164	CuAGAAAuACGuAUUuAcATsT	1165	AD-14376	70%	5%
ucuAGuuuucAuAuAAAGuTsT	1166	ACUUuAuAUGAAAACuAGATsT	1167	AD-14377	49%	4%
AuAAAGuAGuucuuuuAuATsT	1168	uAuAAAAGAACuACUUuAUTsT	1169	AD-14378	46%	3%
ccAuuuGuAGAGcuAcAAATsT	1170	UUUGuAGCUCUuAcAAAUGGTsT	1171	AD-14379	44%	5%
uAuuuucAGuAGucAGAuuTsT	1172	AUUCUGACuACUGAAAAuATsT	1173	AD-14380	35%	16%
AAAucuAAcccuAGuuGuATsT	1174	uACAACuACGGCUuAGAUUUTsT	1175	AD-14381	44%	5%
cuuuAGAGuAuAcAuuGcuTsT	1176	AGcAAUGuAuACUCuAAAGTsT	1177	AD-14382	28%	3%
AucuGAcuAAuGGcucuGuTsT	1178	AcAGAGCcAUuAGUcAGAUTsT	1179	AD-14383	55%	11%
cAcAAuGAuuuAAGGAcuGTsT	1180	cAGUCCUuAAAUcAUGGUGTsT	1181	AD-14384	49%	9%
ucuuuuucucGAuucAAAuTsT	1182	AUUuGAAUCGAGAAAAGATsT	1183	AD-14385	36%	2%
cuuucuuGucGAuucAAAucTsT	1184	GAUUuGAAUCGAGAAAAGTsT	1185	AD-14386	41%	7%
AuuuucuGcucAcGAuGAGTsT	1186	CUcAUCGCGCAGcAGAAAUTsT	1187	AD-14387	38%	3%
uuucuGcucAcGAuGCGuuTsT	1188	AACGcAUCGUGAGcAGAAATsT	1189	AD-14388	50%	4%
AGAGcuAcAAAAccuAucuTsT	1190	GGAuAGGUUUUGuAGCUCUTsT	1191	AD-14389	98%	6%
GAGccAAAGGuAcAccAcuTsT	1192	AGUGGUGuACCUUUGGCUCTsT	1193	AD-14390	43%	8%
GccAAAGCuAcAccAcuACTsT	1194	GuAGUGGUGuACCUUUGGCTsT	1195	AD-14391	48%	4%
GAAcuGuAcucuuucucAGcTsT	1196	GCUGAGAAAGAGuAcAGUUCTsT	1197	AD-14392	44%	3%
AGGuAAAuAucAccAAcAuTsT	1198	AUGUUGGUGAuAUUuACCUTsT	1199	AD-14393	37%	2%
AGcuAcAAAAccuAucccuuTsT	1200	AAGGAuAGGUUUUGuAGCUTsT	1201	AD-14394	114%	7%
uGuGAAAGcAuuuAAuuccTsT	1202	GGAAUuAAAUGCUUUcAcATsT	1203	AD-14395	59%	4%
GcccAcuuuAGAGuAuAcATsT	1204	UGuAuACUCuAAAGUGGGCTsT	1205	AD-14396	49%	5%
uGuGccAcAcucccAAGAccTsT	1206	GCUCUUGGGAGUGUGGcAcATsT	1207	AD-14397	71%	6%
AAAcuAAAuuGAucucGuATsT	1208	uACGAGAUcAAUUuAGUUUTsT	1209	AD-14398	81%	7%
uGAucucGuGAAuuAucuTsT	1210	AGAuAAUUCuACGAGAUcATsT	1211	AD-14399	38%	4%
GcGuGcAGucGGucucccATsT	1212	UGGAGGACCGACUGcACGCTsT	1213	AD-14400	106%	8%
AAAGuuuACGGAcAucuGATsT	1214	UcAGAUGUCUCuAAACUUUTsT	1215	AD-14401	47%	3%
cAGAAGGAAuAuGuAcAAATsT	1216	UUUGuAcAuAUUCCUUCUGTsT	1217	AD-14402	31%	1%
cGcccGAGAGuAccAGGGATsT	1218	UCCCUGGuACUCUCGGGCGTsT	1219	AD-14403	105%	4%
cGGAGCAGAuAGAAcGuuuTsT	1220	AAACGUUCuAUCUGCUCCGTsT	1221	AD-14404	3%	1%
AGAuAGAAcGuuuAAAAcATsT	1222	UGUUUuAAACGUUCuAUCUTsT	1223	AD-14405	15%	1%
GGAAcAGGAAccuuAcAAcTsT	1224	GUuGuGAAGUUCCuGUUCCTsT	1225	AD-14406	44%	5%
GuGAGccAAAGGuAcAccATsT	1226	UGGUGuACCUUUGGCUcACTsT	1227	AD-14407	41%	4%
AuccucccuAGAcuuccccuTsT	1228	AGGGAAGUCuAGGGAGGAUTsT	1229	AD-14408	104%	3%
cAcAcucAAGAccuGuGcTsT	1230	GcAcAGGUCUUGGAGUGUGTsT	1231	AD-14409	67%	4%
AcAGAAGUAAuuGuGcAATsT	1232	UUGcAcAuAUUCCUUCUGUTsT	1233	AD-14410	22%	1%
GuAGAGAcAucuGAcuuuGTsT	1234	cAAAGUcAGAUGUCUCuAATsT	1235	AD-14411	29%	3%
AAuuGAucucGuAGAAuuATsT	1236	uAAUUCuACGAGAUcAAUUTsT	1237	AD-14412	31%	4%

CLAIMS

We claim:

1. A double-stranded ribonucleic acid (dsRNA) for inhibiting the expression of a human Eg5 gene in a cell, wherein said dsRNA comprises at least two sequences that are complementary to each other and wherein a sense strand comprises a first sequence and an antisense strand comprises a second sequence comprising a region of complementarity which is substantially complementary to at least a part of a mRNA encoding Eg5, and wherein said region of complementarity is less than 30 nucleotides in length and wherein said dsRNA, upon contact with a cell expressing said Eg5, inhibits expression of said Eg5 gene,

2. The dsRNA of claim 1, wherein said first sequence is selected from the group consisting of the antisense strand sequences Tables 1-3 and said second sequence is selected from the group consisting of the sense strand sequence of Tables 1-3.

3. The dsRNA of claim 1, wherein said dsRNA comprises at least one modified nucleotide.

4. The dsRNA of claim 2, wherein said dsRNA comprises at least one modified nucleotide.

5. The dsRNA of claim 4, wherein said modified nucleotide is chosen from the group of: a 2'-O-methyl modified nucleotide, a nucleotide comprising a 5'-phosphorothioate group, and a terminal nucleotide linked to a cholesteryl derivative or dodecanoic acid bisdecylamide group.

6. The dsRNA of claim 4, wherein said modified nucleotide is chosen from the group of: a 2'-deoxy-2'-fluoro modified nucleotide, a 2'-deoxy-modified nucleotide, a locked nucleotide, an abasic nucleotide, 2'-amino-modified

nucleotide, 2'-alkyl-modified nucleotide, morpholino nucleotide, a phosphoramidate, and a non-natural base comprising nucleotide.

7. The dsRNA of claim 4, wherein said first sequence is selected from the group consisting of Tables 1-3 and said second sequence is selected from the group consisting of Tables 1-3.

8. A cell comprising the dsRNA of claim 1.

9. A pharmaceutical composition for inhibiting the expression of the Eg5 gene comprising the dsRNA of claim 2.

10. The pharmaceutical composition of claim 9, wherein said first sequence of said dsRNA is selected from the group consisting of sense strand sequences of Tables 1-3 and said second sequence of said dsRNA is selected from the group consisting of the antisense strand sequences of Tables 1-3.

11. The pharmaceutical composition of claim 10 further comprising a dsRNA that inhibits the expression of the VEGF gene.

12. A method for inhibiting the expression of the Eg5 gene in a cell, the method comprising:

(a) introducing into the cell the dsRNA of claim 2; and

(b) maintaining the cell produced in step (a) for a time sufficient to obtain degradation of the mRNA transcript of the Eg5 gene, thereby inhibiting expression of the Eg5 gene in the cell.

13. The method of claim 12 wherein a second dsRNA that inhibits the expression of VEGF is introduced into said cell.

14. A method of treating, preventing or managing pathological processes mediated by Eg5 expression comprising administering to a patient in need of such

treatment, prevention or management a therapeutically or prophylactically effective amount of the dsRNA of claim 2.

15. The method of claim 14 further comprises administering a second dsRNA that inhibits the expression of VEGF.

16. A vector for inhibiting the expression of the Eg5 gene in a cell, said vector comprising a regulatory sequence operably linked to a nucleotide sequence that encodes at least one strand of a dsRNA, wherein one of the strands of said dsRNA is substantially complementary to at least a part of a mRNA encoding Eg5 and wherein said dsRNA is less than 30 base pairs in length and wherein said dsRNA, upon contact with a cell expressing said Eg5, inhibits the expression of said Eg5 gene by at least 40%.

17. A cell comprising the vector of claim 16.